SCREENING NATURAL PRODUCT DATABASE FOR IDENTIFICATION OF POTENTIAL INHIBITORS OF BETA SECRETASE; A KEY ENZYME OF ALZHEIMER'S DISEASE

A Thesis submitted to National Institute of Technology, Rourkela in partial

Fulfillment of the requirements for the award of the degree of

Master of Technology

In

Biotechnology

By

VIVEK KUMAR YADAV

211BM2016

Under the guidance of

Dr. Nandini Sarkar

Department of Biotechnology and Medical Engineering
National Institute of Technology
Rourkela-769008 (ODISHA)
2011-2012

Dr. Nandini Sarkar
Assistant Professor
Department of Biotechnology
and Medical Engineering
National Institute of Technology
Rourkela-769008 (ODISHA)

Phone No.: 0661-2462284
Email: nandini.iitg@gmail.com

CERTIFICATE

This is to certify that the thesis entitled **"SCREENING NATURAL PRODUCT DATABASE FOR IDENTIFICATION OF POTENTIAL INHIBITORS OF BETA SECRETASE; A KEY ENZYME OF ALZHEIMER'S DISEASE"** submitted by **Mr. VIVEK KUMAR YADAV** in partial fulfillment of the requirements for the award of Master of Technology in Biotechnology and Medical engineering with specialization in Biotechnology at the National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Dr. Nandini Sarkar
Dept of Biotechnology and
Medical Engineering
National Institute of
Technology
Rourkela-769008

ACKNOWLEDGEMENTS

I gladly take this opportunity to express my genuine appreciation, gratitude and credit to individuals who have been involved directly or indirectly throughout my project work.

I express my sincere gratitude to **Dr. NANDINI SARKAR**, Department of Biotechnology and Medical Engineering, NIT, Rourkela for her precious advice and guidance throughout the course of my research effort. I also thank her for helping me learning from my mistakes all through the project.

I express my profound respect to **Dr. BIBEKANAND MALLIK,** Department of Life Science, NIT, Rourkela for helping me and being approachable at all the time. I would also thank to all my faculty members, office staffs of Department of Biotechnology & Medical engineering, NIT Rourkela, for their co-operation.

I express deep and sincere thanks to **EKLABYA BISSOYI** for their valuable suggestions and constant encouragement that helped me in successful completion of my project work.

It's my pleasure to thank my friends **ARUN E.V.R., KRISHAN KUMAR, UDAY KUMAR GUPTA, & KAHDE SHANKAR MUKUNDRAO,** and all others for their constant encouragement and for their day-to-day support.

Submitted by
Vivek Kumar Yadav
Dept. of biotechnology and medical engineering
National institute of technology Rourkela
Rourkela- 769008, Odisha
India
Email: vivek.yadav752@gmail.com

ABSTRACT

Alzheimer's disease is a most common amyloid-associated disorder, irreversible, progressive brain disorders that leads to change in nerve cells destroying the thinking, memory, remembering, reasoning and skills of individuals. The symptom of Alzheimer's, disease generally appears in the individuals having age more than 65 years. The neuropathological hallmarks of AD include presence of senile plaques (NP) and neurofibrillary tangles (NFT). Amyloid Beta precursor protein cleaving enzyme (BACE-1) or Memapsin-2 is a single-membrane protein belongs to the Aspartyl protease family, responsible for the processing of the amyloid Precursor protein (APP). The cleavage of APP by BACE-1 leads to production of two peptide fragments Amyloid beta peptide (Aβ) 40 and 42. Aβ42 is thought to be responsible for the neurotoxicity and amyloid plaque formation in Alzheimer's disease (AD). Thus BACE-1 will be an important drug target for the generation of inhibitors that lower Aβ. We aim to identify potential natural product inhibitors of beta secretase which can be further developed as drug to treat Alzheimer's disease. Here, we have performed in silico virtual screening approach of a natural product database consists of 800 different chemical molecules. Beta secretase (PDB ID: 1FKN) was used in screening process, and docking studies to identify potential lead compounds. The sorting of compound were done based upon their binding energy and top 50 ranked protein- inhibitor complexes were selected. We have reported some compound like Spermine, Netilmicinsulfate, and Spermidine Trihydrochloride molecule which could be potential inhibitors of beta secretase. The ligplot image also provided some idea about the inhibitors that they can attain at the active site of beta secretase.

Keywords; BACE-1; Beta secretase; Memapsin; Alzheimer; Amyloid; Aspartyl protease.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

---

1. INTRODUCTION

Alzheimer's disease is a most common amyloid-associated disorder, unalterable, progressive brain disorders that leads to change in nerve cells destroying the thinking, memory, remembering, reasoning and skills of individuals[1]. Alzheimer's disease patient have Insoluble, extracellular amyloid plaques, consist of fibrillar aggregates of the amyloid-beta (Aβ) peptide, a product of the proteolytic cleavage of β-amyloid precursor protein (APP)[2].Two enzymes, γ- secretase and β-secretase (BACE-1 or Memapsin-2), are responsible for the sequential cleavage of APP for the production of Aβ peptide.

Alzheimer's disease is a chronic and progressive neurodegenerative disorder causing dementia in the individuals having age more than 65. The numbers of Alzheimer's disease patients in united states of America are ranging from 3–5 million having an annual estimated cost of approximately $95 billion. The Alzheimer's disease pathological hallmarks consist of presence of extracellular senile plaques and intracellular neurofibrillary tangles (NFT).Because neurofibrillary tangles are intracellular fibrillar aggregates of the microtubule-associated protein tau that exhibit oxidative modifications and hyperphosphorylation[3]. Extracellular senile Plaques and neurofibrillary tangles are present in the regions of brain that involved hippocampus, entorhinal cortex, and basal forebrain. Aβ damages synapses and neurites by interacting with plaques and tangles. Due to which glutamate or acetylcholine neurotransmitters producer neurons are affected mostly, but serotonin and nor-epinephrine neurotransmitters producing neurons damaged.

Generally APP was rapidly cleaved and secreted at high levels and the cleavage done by α- secretase produce residues K16 and L17 of Aβ and generated a secreted derivative, sAPPα, and a membrane bound 83 residue fragment CTFα. The pathway that leading to Aβ

includes the presence of sAPPβ and CTFβ is a membrane bound 99-residue length fragment. The processing of CTFβ and CTFα by γ-secretase yielded Aβ and a smaller 3 kDa fragment called as P3, respectively. Aβ42 plays a very important role in Alzheimer's disease because the mutations occur in familial Alzheimer's disease (FAD) leads to increase in the Aβ42/Aβ40 fragment ratio of peptide, Because these mutations leads to the C-terminal side of the Aβ sequence on APP[4]. Aβ, peptide the intracellular fragment that is produced by the action of γ-secretase processing of CTFα, CTFβ and CTFγ was not detected easily. Aβ plays an important role in Alzheimer's disease pathogenesis arises due to the mutations in the APP genes, PS1 genes and PS2 genes that are responsible for the Alzheimer's disease, leads to increase in production of Aβ42 or total Aβ. Aβ Oligomer are neurotoxic in vitro and in vivo; because they lead to Alzheimer's disease.

Beta Secretase is the key enzyme that are responsible for the production of this potentially toxic peptide so it would be an important drug target for the treatment of Alzheimer's disease. Beta secretase or BACE-1 or Memapsin-2 is a pepsin-like aspartic protease is considered to be a key target for the development of inhibitors of Alzheimer's disease. The drugs those are available for the treatments of Alzheimer's disease have limitations like they have low efficacy, have high cost and have severe side effects. the Small molecule that are obtained from natural products are more significant than compared of chemically synthetic because they work as more potential therapeutic agent against many diseases[5]. Natural compounds may be important in treating the neurodegenerative diseases. So the Beta secretase is a validated drug target for Alzheimer's disease drug discovery, because a particular inhibitor of the enzyme work as a potential drug molecule[6]. Potential inhibitors are identified by Insilco Virtual screening process that bind to beta secretase and the type of interaction and structural differences that exist between the beta secretase and

potential ligand molecule are required to recognize the interaction of potential ligand at the active site of beta secretase with optimization of rational drug candidates.

With the aim of identifying potential natural product inhibitors of beta secretase which can be effective or potential compound for the treatment of Alzheimer's disease we have performed *in silico* virtual screening of a natural product data base that have 800 compounds with different chemical property and entities. Beta secretase (PDB ID: 1FKN) was used in the Insilco virtual screening process, and the docking of compound were performed using Swiss dock server which is based on EADOCK DSS engine (combined with setup scripts for curating common problems and for preparing both the target protein and the ligand input files) considered as one of the best algorithm for small molecular conformational search to identify potential inhibitors molecules[7]. Then the compounds were selected based upon their binding energy and the top 50 compound that have more binding energy have been ranked protein-inhibitor complexes. The protein-inhibitor interactions into clusters and ligplot image of docked compound give some clues on various promising conformations that inhibitors can attain at the active site or bind in near the active site or in binding pocket of active site of beta secretase. After that the small potential molecule that is reported in this study can be further assayed for development into drugs.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. ALZHEIMER, S DISEASE

Alzheimer's disease is a most common amyloid-associated disorder, irretrievable, progressive brain disorders that leads to change in nerve cells destroying the thinking, memory, remembering, and way of thinking and skills of individuals. Alzheimer's disease patient have Insoluble, extracellular amyloid plaques, consist of fibrillar aggregates of the amyloid-beta (Aβ) peptide, a product of the proteolytic cleavage of β-amyloid precursor protein (APP). Alzheimer's disease was first identified more than 100 years ago. Generally Alzheimer's disease appears after the age of 65 years & moreover a most common cause of dementia to old age people.

The Alzheimer's disease was named after Dr. Alois Alzheimer. Dr. Alois Alzheimer noticed changes in the tissue of brain of a woman that died due to mental illness. He found that her brain have many abnormal clumps (amyloid plaques) and tangled bundles of fibers (neurofibrillary tangles). Plaques and tangles are responsible for the failure of brain because they prevent connection between the neurons due to which neuron cannot communicate between them and at last die that lead to Alzheimer's disease. Deposition of Abnormal proteins in the form of amyloid plaques and tau tangles in the brain effect the efficiency of brain mainly in the part of hippocampus that is responsible for the memories[8]. Loss of neuron leads to reduction of brain which is the final stage of Alzheimer's, disease [9, 10]. Alzheimer's disease is misrepresented proteolytic processing of the amyloid beta precursor protein (APP) that results in the production and aggregation of neurotoxic forms of Aβ[11]. Neurons that degenerate in Alzheimer's disease show increased impaired energy metabolism, oxidative damage, and disturbed or agitated cellular calcium homeostasis; so the appearance

of Aβ at this site may be an important instigator to predict these disease forming abnormalities[12].

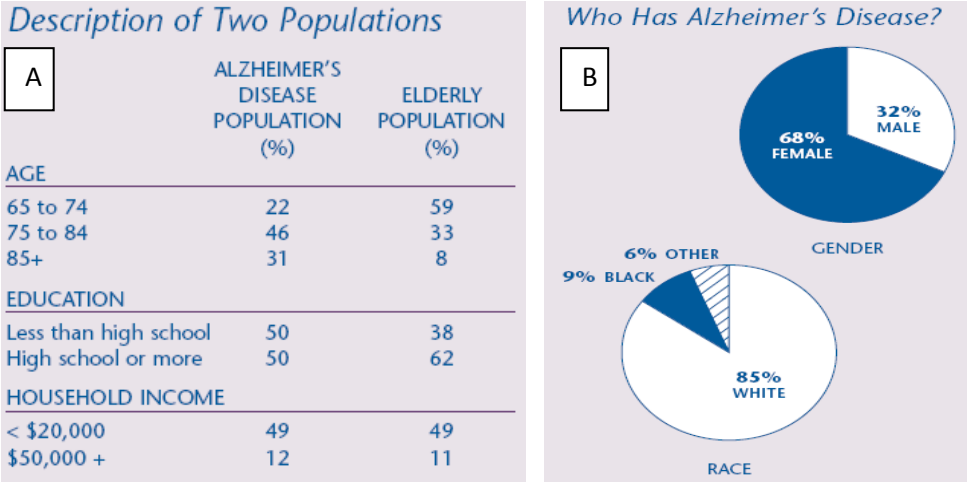


Figure 1: Shows the individual suffering from Alzheimer's disease (a) according to age (b) according to gender & race. [Ref: SOURCE: National Academy on an Aging Society analysis of data from the *1994 National Health Interview Survey of Disability, Phase I.]*

2.1.1. SYMPTOMS OF ALZHEIMER'S DISEASE

Symptoms of Alzheimer's disease causing Dementia that affect mental function, including:

- failure of memory,
- bad temper
- Problems with reasoning and communication
- poignant behavior or qualities
- Thinking and judgment (cognitive skills)
- complexity in solving problems
- Forgetting current events or conversations.
- Taking longer time to perform more complicated activities.

- Alteration in sleep patterns and suddenly wakes up in the night.
- Having Delusions, gloominess, disturbance.
- Complexity doing basic responsibilities, for example preparing meals, selecting suitable clothing, and driving.
- complexity in reading, understanding or writing
- Aggressive behavior underprivileged judgment and failure of ability to recognize danger.
- Using the incorrect statement, mispronouncing terms, speaking in perplexing sentences
- Diminishing himself from social environment and people.

The people suffering with severe Alzheimer's disease can no longer:

- recognize speech
- distinguish family members
- carry out necessary activities of daily living, like eating, dressing, and bathing

A lot of other difficulty that people having this disease faces Incontinence, Swallowing troubles, lapses of memory and have troubles in finding the exact terms[13]. The most important effect of this disease is that public cannot remember information due to which difficulty comes because disturbance of brain neuron function usually involved in forming new memories.

Warning signs of Alzheimer's disease are:

- Mystification with time or place.
- Challenges in planning or solving problems.
- Loss of remembrance that disrupts daily life.
- Complexity carrying out familiar tasks at home, at work or at vacation.
- New troubles with language in communication or writing.

- Difficulty in consideratation of visual images and spatial associations.
- Pulling out from occupation or social activities.
- Changes in mood and character and behavior.
- Misplacing belongings and losing the capability to repeat steps.

2.1.2. CAUSES OF ALZHEIMER'S DISEASE

Amyloid precursor beta protein is a transmembrane protein. It is one of most abundant protein that is present in the central nervous system (CNS) of human[14]. It is also present in peripheral tissue and blood cells along with CNS. Two different pathway to metabolize Amyloid precursor beta protein include

(a) non-amyloidogenic and

(b) Amyloidogenic pathways.

In non- amyloidogenic pathway enzyme γ-secretase slice Amyloid precursor beta protein, into a soluble N-terminal fragment (sAPPα) and a C terminal fragment (C83), additionally cleaving of C terminal domain was done by α-secretase, that discharge a C-terminal fragment of 3KDa (C3)[15, 16]. In Amyloidogenic pathway β-secretase cleaved Amyloid precursor beta protein and releases a smaller N-terminal fragment (sAPPβ) and a C-terminal fragment (C99)[17]. Then γ-secretase cleaves that part and produces the full-length β-amyloid peptides (Aβ).

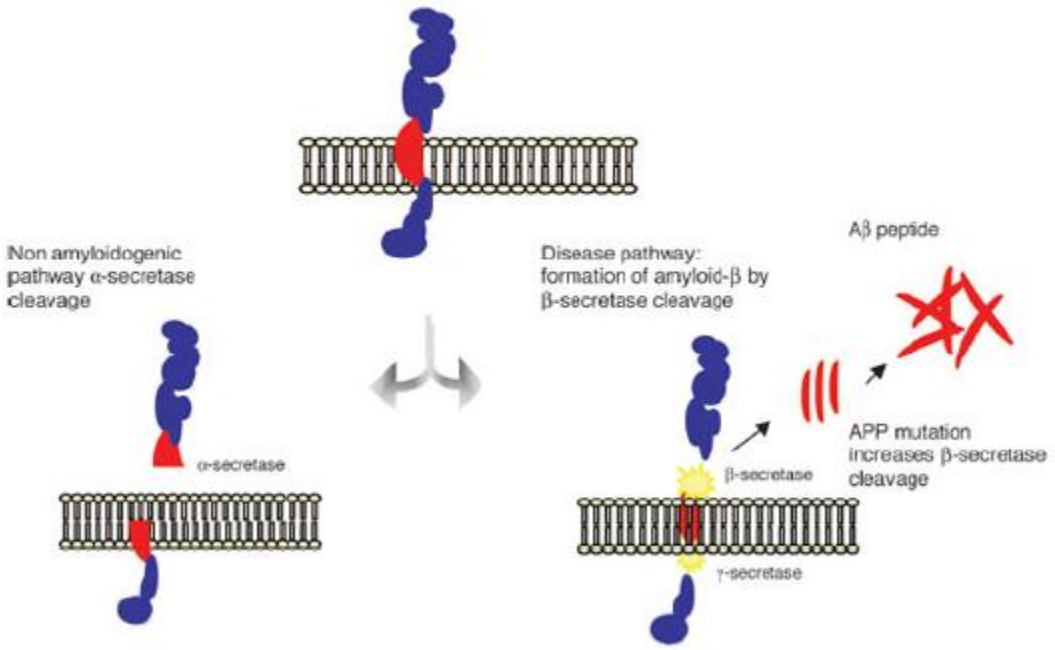


Figure 2: shows non-amyloidogenic pathway and amyloidogenic pathway of sequential cleavage of app by β and γ-secretase.

2.1.2.1. THE AMYLOID-B CASCADE HYPOTHESIS:

The amyloid-β cascade hypothesis was first described by Korczyn in 1990s. According to him gathering of β-amyloid peptides as neurofibrillary tangles and senile plaques in the brain, due to their amplified production or due to the decreased clearance from the brain, is responsible for pathogenetic characteristic of Alzheimer's disease[18, 19]. Due to the accumulation of Aβ peptide many proceedings that affects the neuron occurs like increased oxidative stress inside the neuronal cell, dysfunction of mitochondria, unusual neuroinflammatory response, decreased neuroplasticity ,decreased neurotrophic support, and neurogenesis, hyperphosphorylation of TAU occurs, along with this there are starts of apoptosis and disruption of calcium homeostasis occurs [20]. The Aβ peptide is a little sticky so that they form oligomers; these oligomers interrelate with neurons and microglia cells of brain triggering a sequence of negative events. The amyloid-β cascade hypothesis was based

mostly on *in vivo* studies and *in vitro* studies and then recognition of genetic mutations associated with early-onset of Alzheimer's disease (i.e. mutation in the APP gene, and presenilin 1 and 2 genes)[21]. A mutation in APP genes leads to rise in production of Aβ and aggregation into oligomers, which is deposited as plaques.

2.1.2.2 CHOLINERGIC HYPOTHESIS

According to this hypothesis Alzheimer's disease is caused by reduced synthesis of the neurotransmitter acetylcholine that are accountable for the transmittance of neuronal message from one neuron to another that are associated by synapses[22]. Neurofibrillary tangles formation takes place intracellular and sensile plaques formation takes place extracellular due to which they avoid neuron to communicate between them by forming aggregate of Aβ peptide. Some other cholinergic hypothesis beginning of large-scale aggregation of amyloid, leading to generalized neuroinflammation causing loss of neuron.

2.1.2.3. GSK3β HYPOTHESIS

Aβ peptides and hyperphosphorylated TAU plays a considerable role in the pathogenesis of Alzheimer's disease. According to this hypothesis Neurofibrillary tangles and Aβ sensile plaques are produced by two different mechanism causing Alzheimer's disease. A key enzyme GSK3β regulates cellular metabolism along with including phosphorylation of TAU protein[23]. Wnt signaling leads to the inactivation of GSK3β. usually GSK3β has been found in a hyperactive state that is accountable for hyperphosphorylation of TAU[20]. But in several cases GSK3β also regulates metabolism of amyloid precursor beta protein, and assist in amyloidogenic cleavage leads to overproduction

of Aβ, condensed neurogenesis and increased apoptosis[24]. Activation of GSK3β leads to neuronal changes and loss of neuronal cells that are observed in AD. But the inhibition of GSK3β activity protects in opposition to neuronal degeneration and death induced by Aβ and Tau hyperphosphorylation.

2.1.3. PATHWAY OF ALZHEIMER'S DISEASE

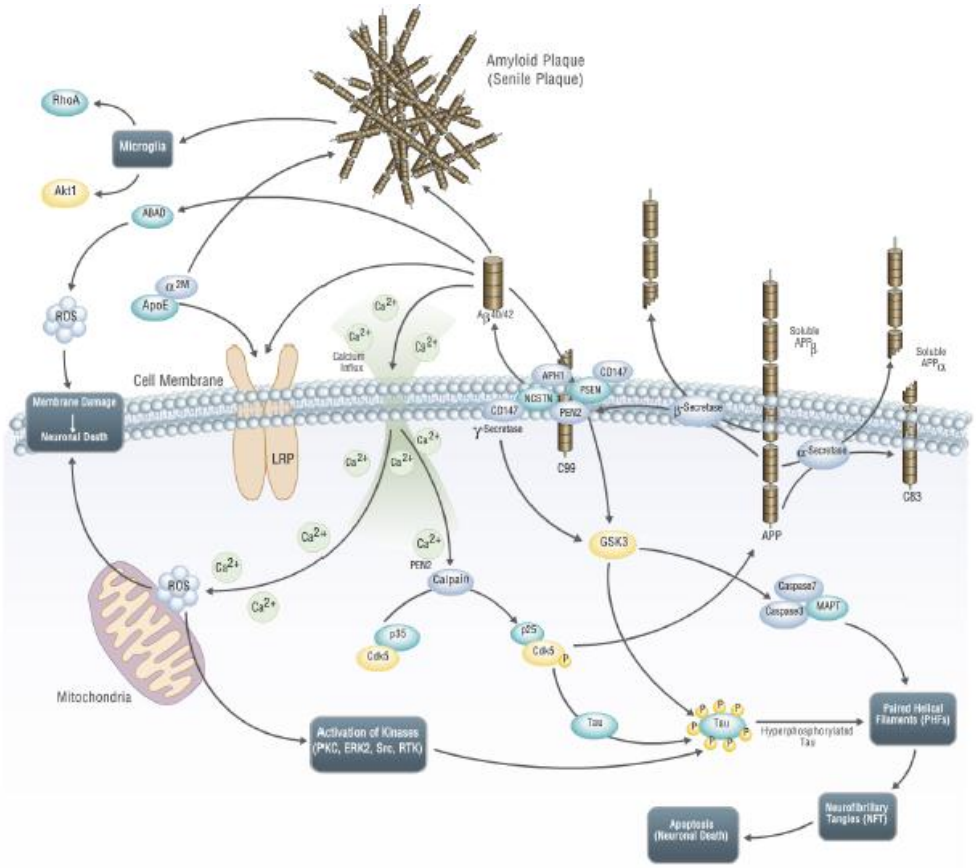


Figure 3: shows production of Aβ peptide and their fate in different compartment of cell.

The above figure shows the sequential production of Aβ peptide from the APP protein by different sequential cleavage by different enzymes including alpha secretase, beta secretase, and gamma secretase.

2.2 ROLE OF BETA SECRETASE IN ALZHEIMER'S DISEASE

BACE-1 or β-secretase, memapsin-2, or Aspartyl protease-2, is a single-membrane protein belongs to the Aspartyl protease class of enzyme accountable for the cleavage of app[25]. The cleavage of amyloid Beta precursor protein by beta secretase, consequences in the production of two peptide fragments Aβ40 and Aβ42[26]. Aβ42 is the most important species and are accountable for the neurotoxicity and amyloid plaque development in the brain that lead to Loss of neuron in the cortex and hippocampus section, guide to loss of memory and numerous defects in Alzheimer's disease. Thus the Inhibition of beta secretase has emerged as an attractive therapeutic objective for Alzheimer's disease[27]. During 1999–2000, five teams concluded that the novel transmembrane Aspartyl protease BACE-1 (also named Memapsin-2 and Asp2) was the β-secretase. BACE-1 has two aspartic protease active site motifs, DTGS (residues 93–96) and DSGT (residues 289–292); BACE- 1 has an N-terminal signal sequence (residues 1–21). Beta secretase has a single transmembrane domain near its C-terminus (residues 455–480) and a palmitoylated cytoplasmic tail.

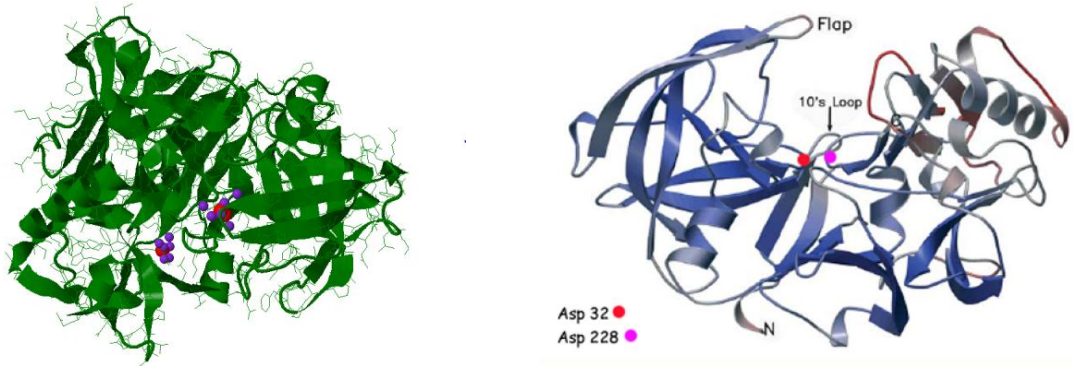


figure 4: Ribbon diagram of beta secretase (PDB.ID 1FKN) showing active site containing Asp residues at 32 and 228 position on amino acid length of protein[REF; protopedia life in 3d interactive encyclopedia of proteins, RNA, DNA and other molecule] .

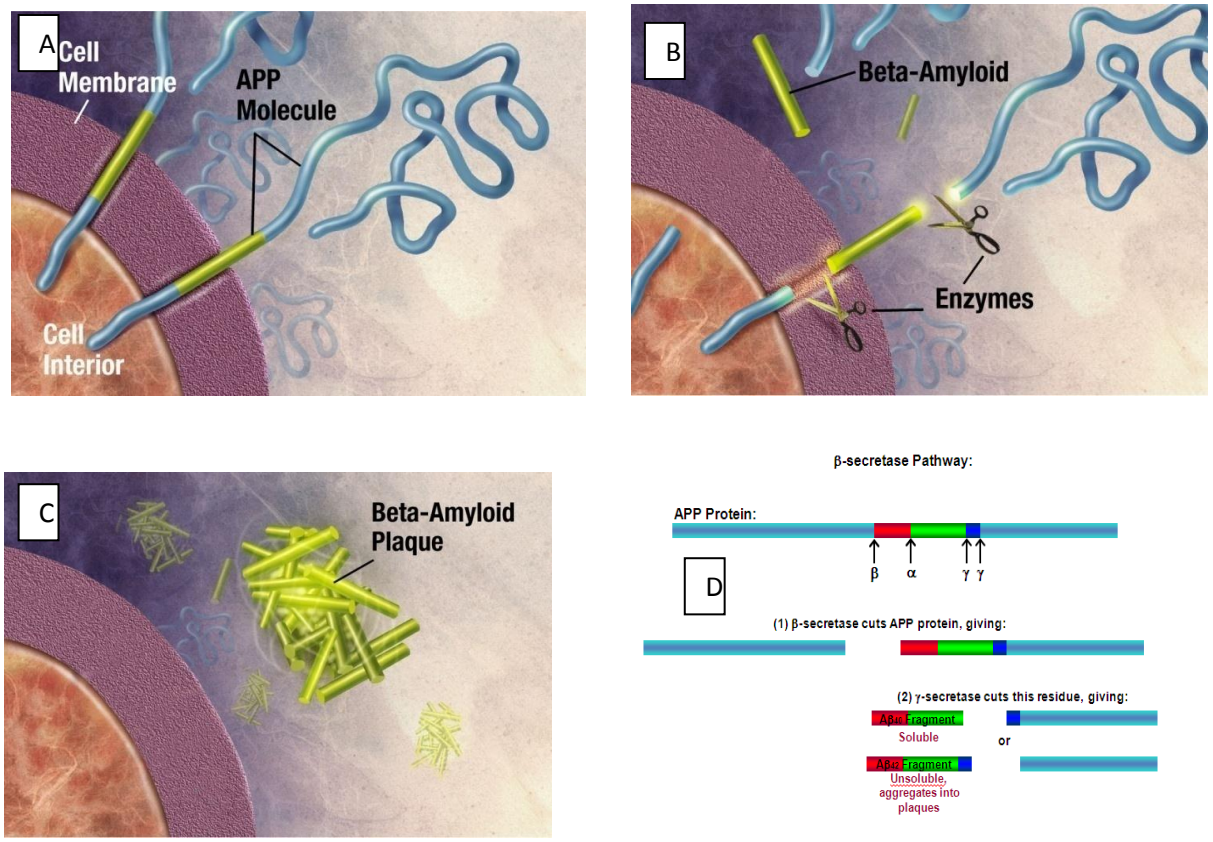


Figure 5: generation of Aβ peptide by beta secretase *(*pdb.id 1fkn) (A to C). D shows sequential cleavage of app. [From: www.niapublications.org/pubs/unraveling/01.htm]

β- Secretase cleaves Aβ at its N-terminus because of nucleophilic attack occurs at the active site of β-secretase. This β-site area which β-secretase binds to is made up of methionine-aspartate-alanine (MDA) residues. The aspartates molecule presents on β-secretase combine to the aspartate residues of the β-site on APP (Asp672). This reaction is positive under acidic conditions that forcing N-terminus residues to split its bond with sAPPβ. The loop present in beta secretase assists in stabilization of protein with APP. If loop is absent then β-secretase is not capable to stabilize its interaction with APP and cleavage of APP will not be done. Active site of β- secretase is made up of two aspartate residues: Asp32 and Asp228. The R groups of both the aspartates residues allowing for a nucleophilic attack to take place on the carbonyls[28]. So the molecule that have the resemblance more than APP

and having possessions to bind with the aspartate residues present at the active site of beta secretase may inhibit the enzyme to bind with the APP, may serve as a potential inhibitors of beta secretase.

2.3. CURRENT THERAPEUTIC STRATEGY

In present time there is no known treatment are available for treatment of Alzheimer's disease. But with the help of medications we can control the agitation, depression or psychotic behavior.

Currently five drugs are available that are approved by FDA and can be used as for the cure of Alzheimer's disease. The Medicines are: Exelon® (rivastigmine); Cognex® (tacrine); Razadyne® (galantamine) and Aricept® (donepezil); these medicines help in inhibition of cholinesterase enzyme that is work as a neurotransmitter in synapses to assist neuron to communicate between them, also help in regulation and interruption of symptoms of Alzheimer's disease[29]. The individuals that are suffering from Alzheimer's disease have very low levels of acetylcholine hormones, so these drugs help in communication between neuron. Acetyl cholinesterase inhibitors assist in sustain the rate at which acetylcholine hormone is broken and thereby increasing the concentration of acetylcholine in the brain uphold the balance of loss occurs due to death of cholinergic neurons[30]. These drugs also slowing degeneration of neuron and help in enhancement of function of brain also help in maintaining judgment, speaking skills, and remembrance, and also with certain behavioral problems. But the one thing is that these medicines have a few side effects like headache, decreased heart rate (bradycardia), loss of weight, decreased hunger, cramps take place in muscles, exhaustion, vomiting and production of increased gastric acid in elevated amount. The U.S. Food and Drug Administration approved a new drug for the treatment of

Alzheimer's disease in 2006 was Namenda® (memantine) used to treat moderate Alzheimer's disease. These drugs are administered orally. But when these drugs present in brain in surplus amount leads to death of neuronal cell, a process called excitotoxicity[31]. Excitotoxicity leads to manufacture of large amount of glutamate as of overstimulation of glutamate receptors. Antipsychotic drugs are also helpful in treatment of Alzheimer's disease by dropping aggression and obsession in individuals suffering from this disease[32]. In this study we aim to identify potential natural product inhibitors of beta secretase which can be additionally developed as drug to treat Alzheimer's disease. BACE-1 will be a significant drug target for the production of inhibitors that lower Aβ. Here; we have performed in silico virtual screening approach of a natural product database consists of 800 different chemical molecules. Beta secretase (PDB ID: 1FKN) was used in screening process, and docking studies to identify potential lead compounds. The categorization of compound were done based upon their binding energy, and there type of interaction with the active site of beta secretase and top 50 ranked protein- inhibitor complexes were selected.

CHAPTER 3

OBJECTIVES

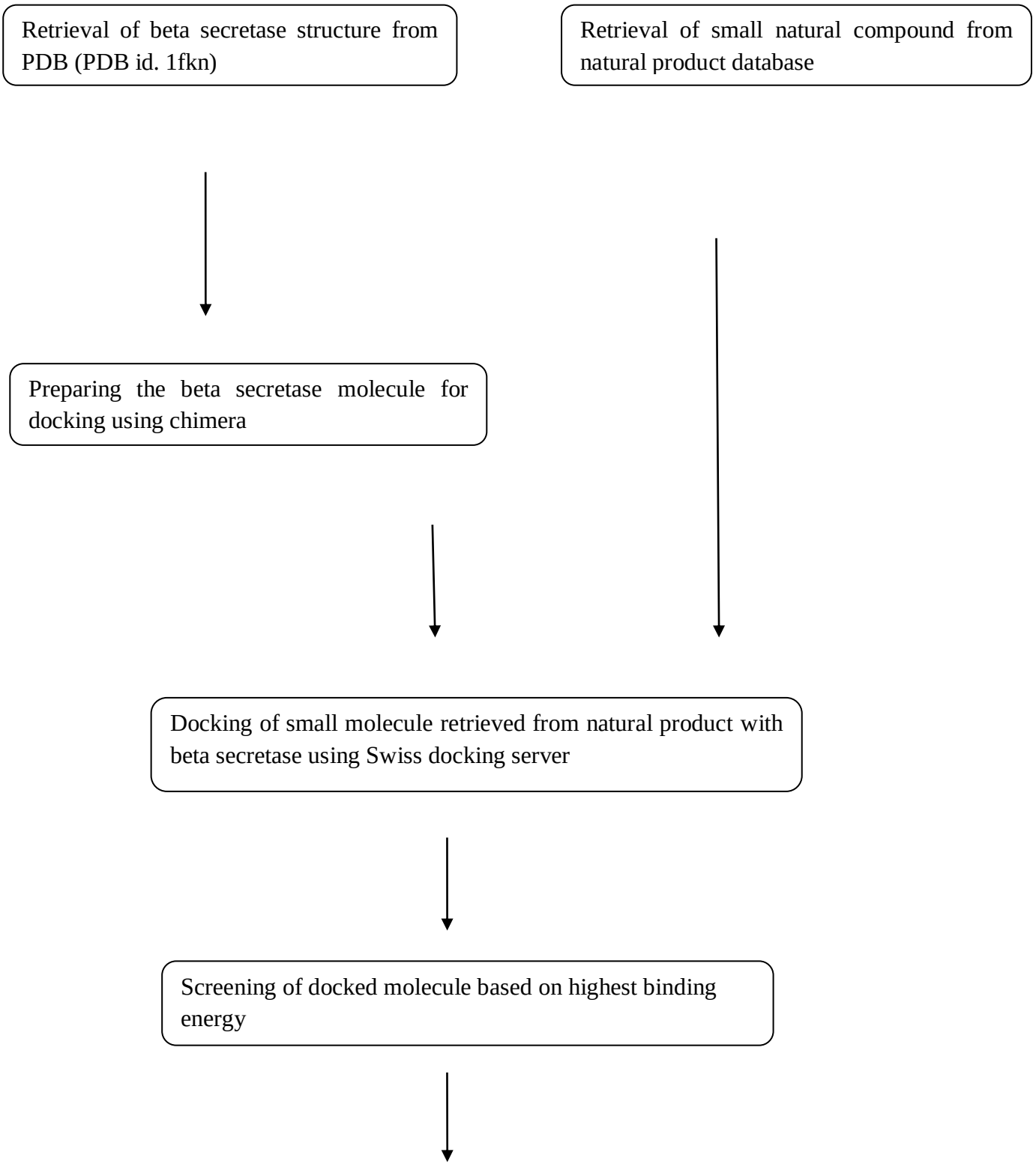
3 OBJECTIVES

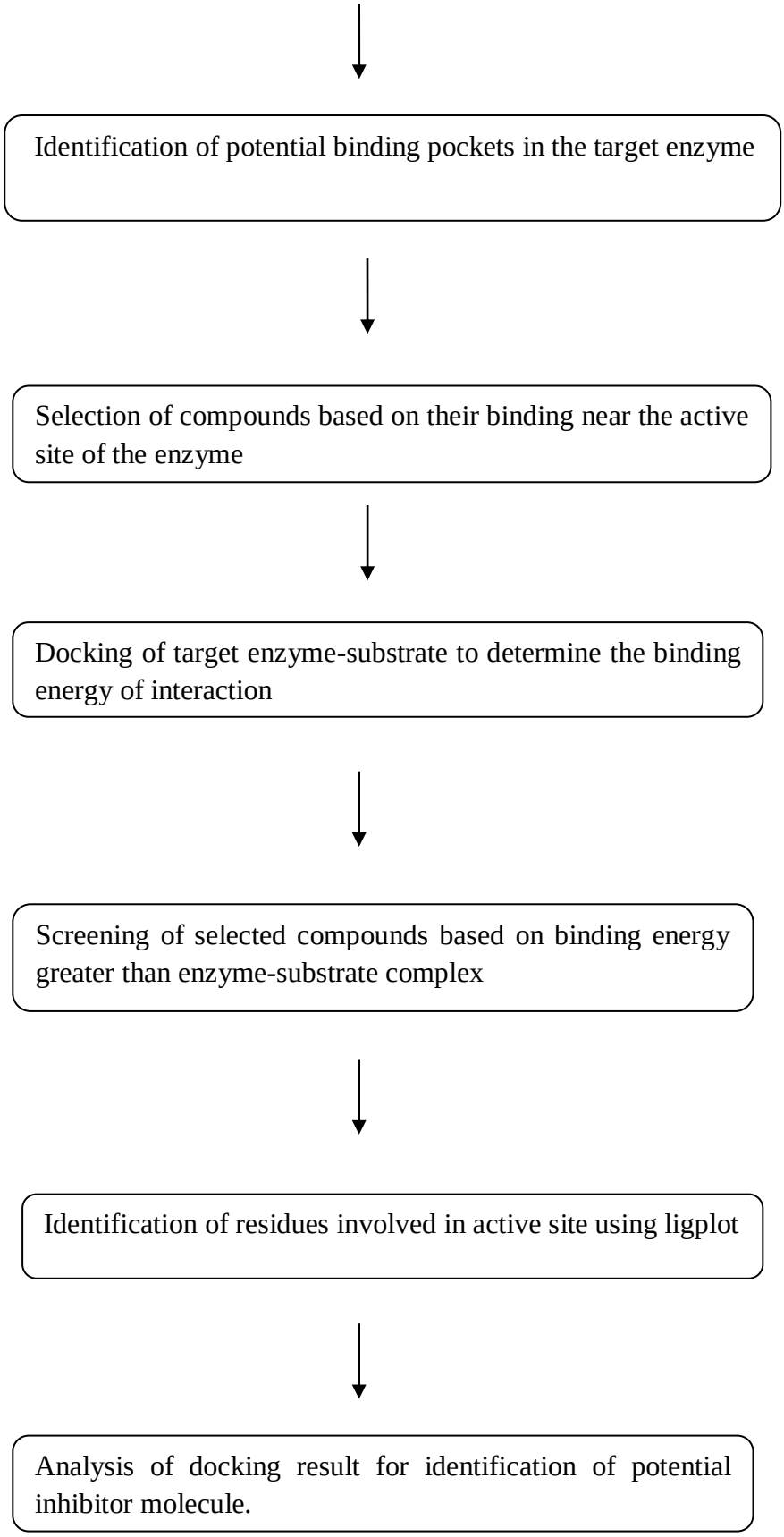
- Virtual screening of compound from natural product database.
- Finding the best potential inhibitors based on energy calculations of their interactions.
- Identification of potential binding pockets in the target enzyme.
- Screening of selected compounds based on their binding near the active site of the enzyme.
- Target enzyme-substrate docking to determine the binding energy of interaction.
- Screening of selected compounds based on binding energy greater than enzyme-substrate complex.
- Analysis of the docking result for identification of potential inhibitor molecule.

CHAPTER 4

PLAN OF WORK

4 PLAN OF WORK




Identification of potential binding pockets in the target enzyme
Selection of compounds based on their binding near the active site of the enzyme
Docking of target enzyme-substrate to determine the binding energy of interaction
Screening of selected compounds based on binding energy greater than enzyme-substrate complex
Identification of residues involved in active site using ligplot
Analysis of docking result for identification of potential inhibitor molecule.

CHAPTER 5

MATERIALS AND METHODS

5 MATERIALS AND METHODS

5.1 MATERIALS:

5.1.1 Requirement of files:

- PDB file of beta secretase (PDB ID 1FKN).
- Mol 2 file or ZINC AC file of ligand molecule.
- CHIMEX files for input into UCSF chimera.
- PDB file of docked molecule for the identification of type of interaction into ligplot.

5.1.2 Requirement of Software's:

- Chimera 1.6.1
- Hex software 6.1
- Ligplot

5.1.3 Requirement of online server's:

- http://www.rcsb.org/
- http://www.msdiscovery.com/natprod.html
- http://www.swissdock.ch/
- http://projects.biotec.tu-dresden.de/metapocket/
- http://dogsite.zbh.uni-hamburg.de/

5.2 METHODS:

In this Insilco based screening, we have selected beta secretase (PDB ID 1FKN) which plays a critical role in the development of amyloid beta peptide that are responsible for main cause of Alzheimer's disease.

5.2.1 Preparation of protein molecule:

Beta secretase (PDB ID 1FKN) were downloaded from the protein data bank (http://www.rcsb.org/pdb), already complexed with the inhibitors. Beta-secretase is a membrane-associated aspartic protease made up of two chain A& B, 391 long amino acid sequence protein. Then this PDB file of beta secretase is opened in chimera 1.6.1 where the inhibitor molecule that is complexed with beta secretase was removed and we get pure form of beta secretase. Then pdb file of this molecule was saved for further use in docking process.

5.2.2 Selection of ligand molecule:

Ligand molecule was selected from natural product database (http://www.msdiscovery.com/natprod.html), containing 800 chemical entities obtained from different natural sources. These entities have flavonoids, alkaloids, phenones, terpenes, chalones, sterols, comurins compound some obtained from plant and microbes.

5.2.3. Docking:

In this study we have used swissdock server (http://www.swissdock.ch/) for protein-ligand docking. It is an online web server based on EADock DSS, used for protein ligand docking.

5.2.3.1. Inputs:

In swissdock server we have to follow just three steps to access docking. The sample files can be directly uploaded into the form simply by clicking on a provided link in the swissdock server.

5.2.3.1.1. Selection of target protein:

Target protein was loaded by just providing the PDB ID or protein FASTA sequence or by URL or by uploading as the PDB files that was saved earlier after processed in chimera. When we uploading the target protein file it takes few seconds to upload because between that time server processed the uploaded protein by calculating on the basis of CHARMM force field.

5.2.3.1.2. Selection of ligand molecule:

Ligand molecule can be selected either by giving ligand name or by ZINC accession code or by categories like scaffolds, side chains or by providing URL or by preprocessed ligand molecule as mol2 file.

5.2.3.1.3. Docking parameters:

According to our requirement we can select docking parameters like type of docking that to start between uploaded ligand and target molecule like fast, accurate or very fast. Then we have selected accurate mode of docking that take desired docking time, number of

minimization steps to relax ligand and number of predicted binding modes. Select the region that containing the active site/binding site of beta secretase to prevent whole scanning of ligand molecule for docking to target protein.

5.2.3.2. Outputs:

When the docking is completed the output file is downloaded from the link provided by the web server in the form of CHIMEX format after a docking assay has been submitted, The docking result web page gives image of docked molecule in JMOL form. From that we can visualize or predict the mode of binding of ligand molecule with the target protein. We can also visualize this mode of binding by opening the downloaded CHIMEX file into UCSF chimera. Web page also provide result in zip file format that contain PDB file of docked complex of target with ligand and also contains mode of binding in dock format.

5.2.4. Prediction of binding site of protein:

Binding site of beta secretase was predicted by mate pocket 2.0 (http://projects.biotec.tu dresden.de/METAPOCKET/). Mate pocket 2.0 is an online server used for prediction of number of binding site/active site present in a protein by using different active site prediction tools like Q site finder, LIGSITE, PASS11, POCASA, FPOCKET, SURFNET, GHECOM, ConCAVITY. There are 10 different binding pockets are present in the beta secretase to which the ligand molecule bind. Meta pocket 2.0 predicted the top three potential binding pockets containing amino acid residues, all atoms that are present in the beta secretase. We can visualize the protein structure and binding mode in JMOL format.

Active site of beta secretase contains two aspartate residues situated at position 32 and 228 position of amino acid sequence.

5.2.5. Analysis and active site prediction:

The active site/ binding site of beta secretase was also predicted by DogSiteScorer (http://dogsite.zbh.uni-hamburg.de/). It is used for analysis and prediction for protein druggability. DoGSite predicted that beta secretase is made up of 10 pockets and 14 different sub pockets, also predict the size, shape, global properties, and chemical features of pockets, volume, depth, surface, residues, as well as functional groups present in the pockets, and give a Simple Score for each pocket between zero and one to predict druggability. According to this prediction the molecule that has score close to one having the property to be worked as potential inhibitors of beta secretase.

5.2.6. Structural analysis of docked molecule:

The binding energy of docked molecule was obtained from web server of swissdock by just clicking on the link provided on the bottom of web server and then the saved CHIMEX file was opened into chimera for the structural analysis of docked molecule. By using UCSF chimera we convert the CHIMEX file into PDB file to understand mode of interaction between the target and ligand molecule.

5.2.7. Ligplot

Ligplot is software, used to study interactions between the protein and the ligand molecule in the protein-ligand complex. Saved PDB file of docked molecule from UCSF chimera was opened into Ligplot by following these steps.

- Open the PDB file saved from UCSF chimera.
- Select the protein and ligand chain.
- Go to run button

The image shows type of interaction like hydrogen bonding, hydrophobic interaction to or near the active site of protein.

CHAPTER 6

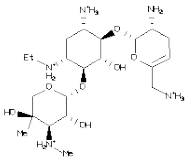
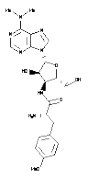
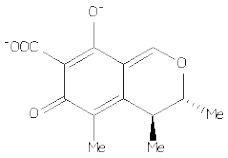
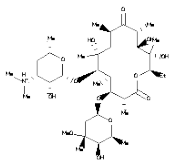
RESULTS

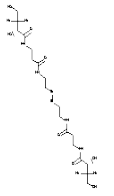
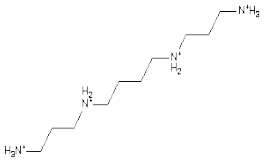
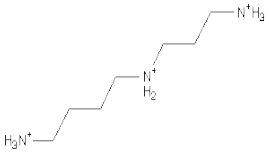
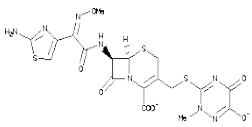
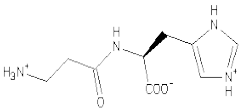
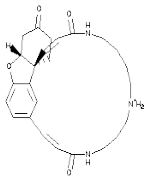
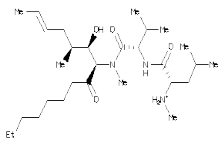
6 RESULTS

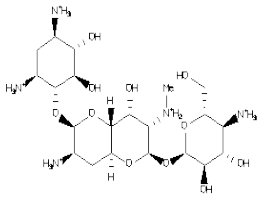
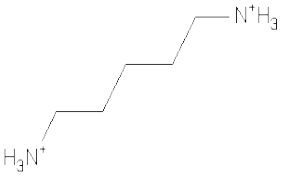
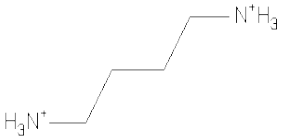
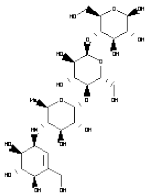
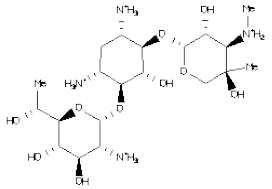
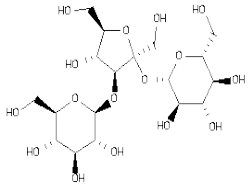
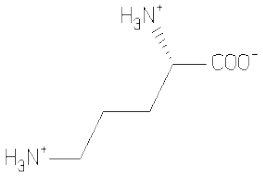
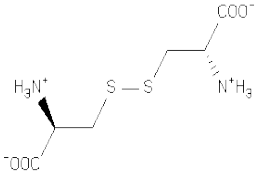
In this study beta secretase (PDB ID 1FKN) was docked with 800 compound obtained from natural product database. The results of structure based virtual screening revealed the mode and type of interaction of ligand molecule with the beta secretase (target) protein molecule. Here we have selected molecules based on their binding energy.

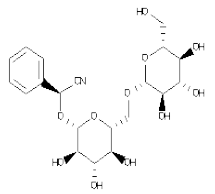
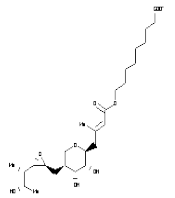
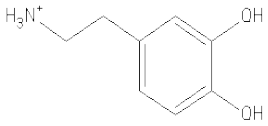
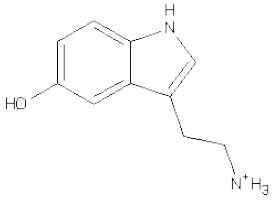
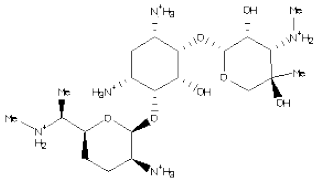
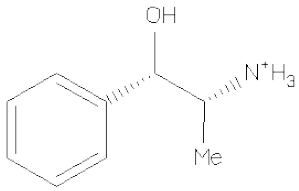
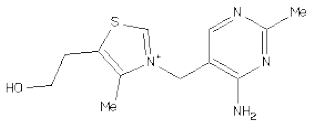
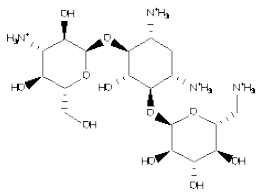
6.1. BEST POTENTIAL INHIBITORS BASED ON ENERGY CALCULATIONS OF THEIR INTERACTIONS.

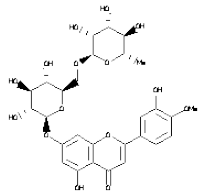
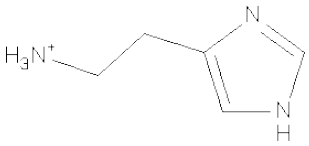
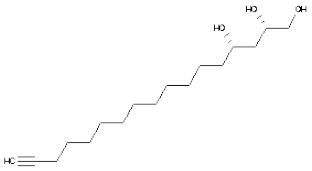
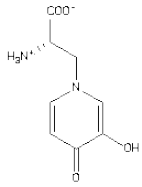
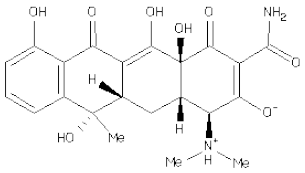
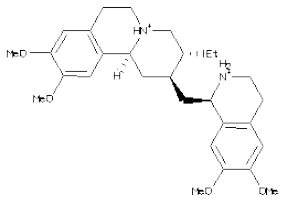
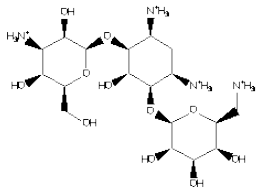
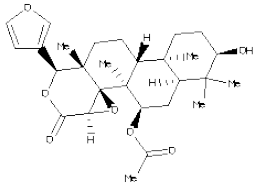
Table 1 shows the structure properties and docking statics of top 44 compounds.

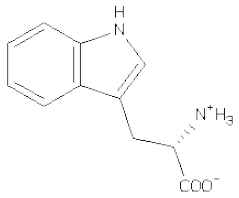
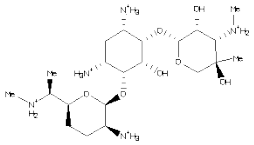
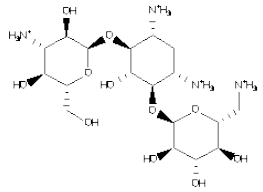
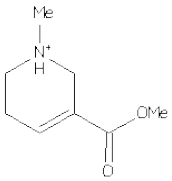
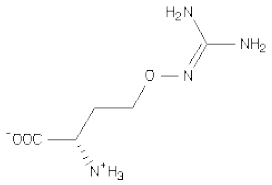
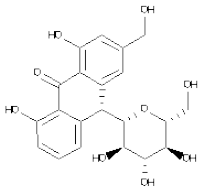
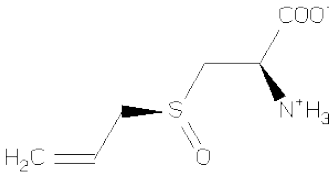
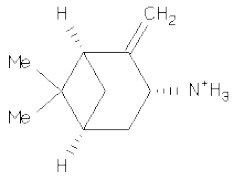
Sr. no.	Name of compound	Structure	Source	Mol. wt.	Binding energy
1.	NETILMICIN SULFATE		semisenthic (sch-20569)	671.7354	-11.51
2.	PUROMYCIN HYDROCHLORIDE		*streptomyces alboniger*	544.4423	-11.31
3.	ANTIMYCIN A		*Streptomyces spp*	534.6118	-11.27
4.	ERYTHROMYCIN		*Streptomyces erythreus*	733.9454	-10.99

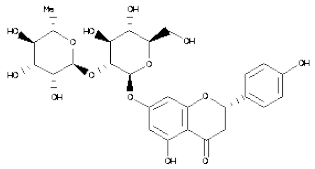
5.	PANTETHINE		semisynthetic	554.73	-10.96
6.	SPERMINE		all animal tissue, fungi	202.3455	-10.92
7.	SPERMIDINE TRIHYDROCHLORIDE		*animal tissue and coprinus comatus*	254.6325	-10.78
8.	CEPHALOSPORIN C SODIUM		*cephalosprium acremonium*	427.4039	-10.66
9.	CARNOSINE		mammalian skeletal muscle	226.2369	-10.36
10.	LUNARINE		*lunaria spp*	437.5435	-10.26
11.	CYCLOSPORINE		*tolypocladium inflatum*	1202.642	-10.03

12.	APRAMYCIN		*streptomyces tenebrarius, saccharomyces porispora hiltus*	539.5878	-10.01
13.	CADAVERINE TARTRATE		putrifaction of lysine	224.2154	-9.75
14.	PUTRESCINE DIHYDROCHLORIDE		catabolism of ornithine	161.0756	-9.55
15.	ACARBOSE		*actinoplanes spp*	645.6174	-9.52
16.	GENETICIN		*micromonospora species*	692.7177	-9.43
17.	MELEZITOSE		honey & plant exudates	504.4461	-9.42
18.	ORNITHINE		widespread in nature	132.1636	-9.38
19.	CYSTINE		widespread in plants and animals	240.3015	-9.37

20.	AMYGDALIN		*rosaceae spp*	457.4383	-9.23
21.	MUPIROCIN		*pseudomonas fluorescens; brl-4910a*	500.6352	-9.21
22.	3- HYDROXYTYRAMINE		synthetic	153.1824	-9.13
23.	SEROTONIN HYDROCHLORIDE		CNS, GI tract, all animals, many mushrooms & plants	212.6809	-9.04
24.	GENTAMICIN SULFATE		*micromonospora spp*	575.6837	-9.03
25.	1R,2S PHENYLPROPYLAMINE		*ephedra vulgaris (mahuang)*	151.2101	-8.98
26.	THIAMINE		rice husks, wheat germ, yeast	337.2735	-8.94
27.	BEKANAMYCIN SULFATE		*semisynthetic; streptomyces kanamyceticus; nk-1006*	581.6006	-8.93

28.	DIOSMIN		*zanthoxylum avicennae*	608.5582	-8.92
29.	HISTAMINE DIHYDROCHLORIDE		*claviceps purpurea*	184.0695	-8.56
30.	AVOCADYNE		*persea spp*	284.4428	-8.38
31.	MIMOSINE		*Mimosa and Leucena spp*	198.1799	-8.33
32.	TETRACYCLINE HYDROCHLORIDE		*streptomyces spp*	480.9062	-8.25
33.	EMETINE		*uragoga ipecacuanha*	553.5751	-8.23
34.	KANAMYCIN A SULFATE		*streptomyces kanamyceticus*	582.5854	-8.23
35.	alpha-DIHYDROGEDUNOL		*derivative*	486.6109	-8.23

36.	TRYPTOPHAN		many plants, animal protein	204.2305	-8.21
37.	GENTAMICIN SULFATE		*micromonospora spp*	575.6837	-8.19
38.	BEKANAMYCIN SULFATE		*semisynthetic; streptomyces kanamyceticus; nk-1006*	581.6006	-8.17
39.	ARECOLINE HYDROBROMIDE		*betel nuts (arica catechu)*	236.1103	-8.12
40.	CANAVANINE		*canavalia ensiformis*	176.1764	-8.10
41.	ALOIN		aloe	434.4035	-8.09
42.	ALLIIN		*allium species*	177.2235	-8.09
43.	3-AMINO-beta-PINENE		*derivative*	187.7147	-8.09

44.	NARINGIN		*citrus spp*	580.5477	-8.06

6.2. IDENTIFICATION OF POTENTIAL BINDING SITE IN THE TARGET ENZYME:

Active site of beta secretase is made up of two aspartate residues situated as position 32 and 228 of the amino acid sequence of protein. Active site/ binding site of beta secretase were predicted by METAPOCKET 2.0 servers and DOGSITE scorer server. METAPOCKET 2.0 is an online server where we have uploaded PDB file of beta secretase then it gives the top three potential binding sites containing amino acid residues that are present to the active site or near the active site of target protein, it also tells all no. of amino acid present in each of top three potential binding sites. METAPOCKET 2.0 servers uses 10 active site prediction tools including Q site finder, pass11, LigsiteCS, GHECOM, POCASA, Fpocket, SURFNET, ConCavity and predict top three potential binding pockets.

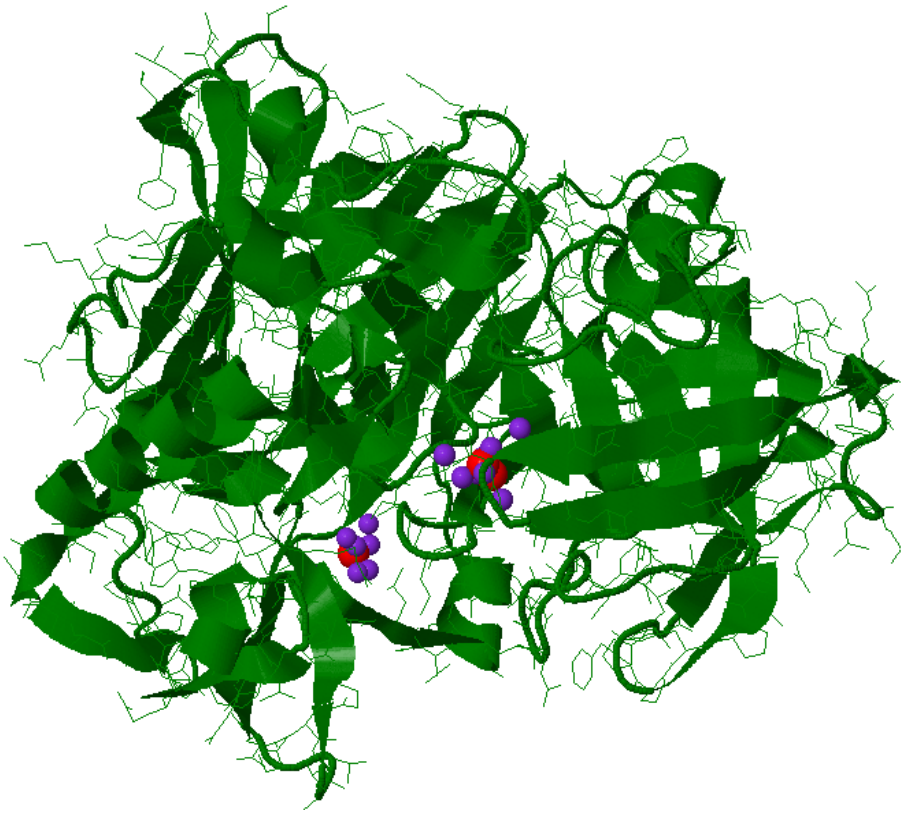


Figure 6: show JMOL view of protein structure and binding sites (pdb.id 1fkn) predicted by METAPOCKET 2.0.

The above image belongs to beta secretase predicted by METAPOCKET 2.0 shows pockets (binding sites) and sub pockets. Active site of beta secretase contains Asp 32 and Asp 228(fig.8.). The β hairpin loop over the active site, known as the "flap "and the 10s loop also contains within it a glycine residue (gly11) with which the substrate can form a hydrogen bond.

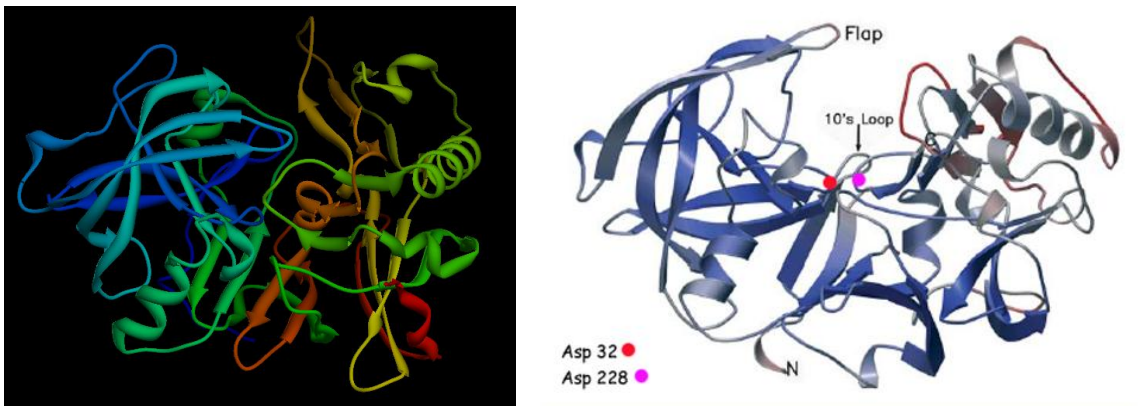


Figure 7: Ribbon diagram of beta secretase (pdb.id 1fkn) showing active site containing Asp residues at 32 and 228 positions on amino acid length of protein.

Binding site of beta secretase was also predicted by DOGSITE scorer, an online server used for structural analysis and active site prediction of beta secretase, also used to check protein druggability. DoGSite predicted that beta secretase is made up of 13 different pockets (binding site) and 10 different sub pockets, also predict the size, shape, global properties, and chemical features of pockets, volume, depth, surface, residues, as well as functional groups present in the pockets, and give a Simple Score for each pocket between zero and one to predict druggability. According to this prediction the molecule that has score close to one having the property to be worked as potential inhibitors of beta secretase. From this table the ligand molecules that have score close to one and those bind to the active site or near the active site can be serving as potential inhibitors of beta secretase.

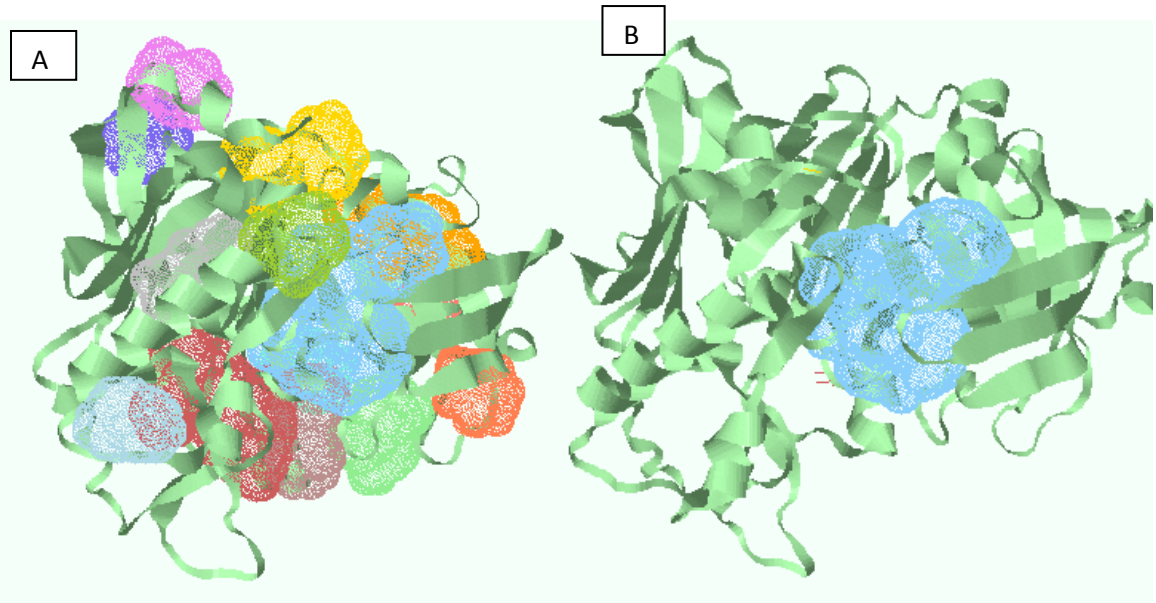


Figure 8: DoGSite predicted (a) all pockets and (b) only P_O present in beta secretase (PDB id 1fkn).

Table 2: Illustrates All Binding Site (Pockets) and Their Drug Score of Beta Secretase (PDB Id 1fkn).

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	877.50	919.97	500.16	21.41	0.82
P1	542.40	687.59	421.62	18.08	0.79
P2	226.88	443.50	242.27	12.98	0.51
P3	184.77	158.75	81.00	15.78	0.59
P4	167.68	310.94	97.12	12.22	0.39
P5	142.08	285.45	173.04	9.02	0.28
P6	139.20	239.22	146.99	12.96	0.41
P7	129.60	206.32	144.79	9.07	0.29
P8	124.74	80.70	77.90	9.37	0.31
P9	121.47	201.42	76.39	7.81	0.19
P10	114.56	224.80	112.17	7.82	0.23
P11	109.06	247.04	183.08	8.10	0.21
P12	100.80	266.13	173.26	6.65	0.13
P13	100.42	246.37	181.35	7.47	0.21

Table 3: Illustrates all sub pockets and their drug score of beta secretase (pdb id 1fkn).

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0SP0	834.18	906.44	490.34	15.74	0.63
P0SP1	43.33	72.47	54.73	0.40	0.39
P1SP0	437.95	559.00	326.39	18.08	0.42
P1SP1	104.45	247.19	176.39	9.24	0.14
P2SP0	165.82	361.27	204.05	0.69	0.22
P2SP1	61.06	194.54	107.37	6.21	0.09
P3SP0	125.18	132.54	69.91	10.23	0.31
P3SP1	59.58	63.11	27.48	8.59	0.21
P4SP0	111.30	278.70	78.27	7.77	0.09
P4SP1	56.38	90.10	28.61	1.26	0.23

6.3. SCREENING OF SELECTED COMPOUNDS BASED ON THEIR BINDING NEAR THE ACTIVE SITE OF THE ENZYME:

By following METAPOCKET 2.0 and DoGSite scorer we have selected following compound that bind to the active site or near the active site of beta secretase. These compounds comes in top three binding pockets having druggability score close to one and bind to the active site or near the active site of beta secretase enzyme.

Table 4: Illustrate ligand molecule bind to or near the active site of beta secretase

Sr.No.	Molecule bound to both asp 32 & asp 228	molecule bound to either asp 32 or asp 228	molecule bind near the active site	molecule bind near the active site by hydrophobic interaction
1.	SPERMINE	3- AMINO BETEPIENENE	ACARBOSE	ALLOIN
2.	SPERMIDINE HYDROCHLORIDE	ARECOLINE HYDROBROMIDE	AMYGDLIN	Alpha-DIHYDROGLUDENOL

3.	3- HYDROXY TYRAMINE	THIAMINE	ANTIMYCIN A	APEGENIN
4.	1R,2S PHENYLPROPYLAMINE		APRAMYCIN	BEKANAMYCIN SULPHATE
5.	TRYPTOPHAN		ATROPINE SULPHATE	CITRININ
6.	SEROTONIN HYDROCHLORIDE		AVOCADYNE	CRUSDESONE
7.	PUTRESEDINE HYDROCHLORIDE		CANVELATOXIN	DIFLUNISAL
8.	MIMOSINE		CENEIOLE	ERGOSTEROL
9.	CYSTINE		CYCLOSPORINE	GENTAMYCIN SULPHATE
10.	CARNOSINE		CEPHALOSPORIN C SODIUM	KANAMYCIN SULPHATE
11.	CANAVENINE		DIOSMIN	PUROMYCIN HYDROCHLORIDE
12.	CADAVARINE TARTERATE		EMETINE	
13.			ERYTHROMYCIN	
14.			GENETECIN	
15.			LUNARINE	
16.			MELEZITOSE	
17.			MUPIROCIN	

18.			NARINGIN	
19.			NELEMICIN SULPHATE	
20.			ORNITHINE	
21.			PANTETHINE	
22.			QUERCETIN	
23.			TETRACYCLINE HYDROCHLORIDE	

6.4. DOCKING OF TARGET ENZYME-SUBSTRATE:

Bate secretase (PDB ID 1FKN) were docked with amyloid beta precursor protein E2 binding domain (PDB ID 3NYL) by using hex 6.1 tool, and binding energy was calculated. Then the pdb file was opened into chimera to visualize the type of interaction between the target protein and ligand molecule. Binding energy calculated by hex was – 8.84.

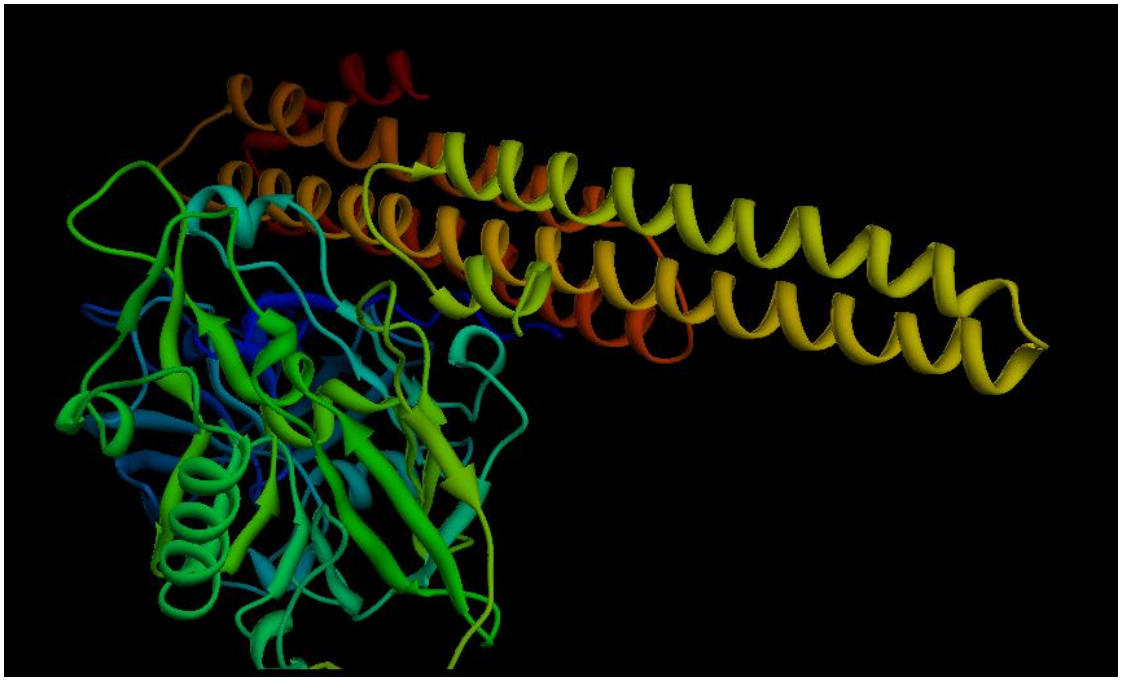


Figure 9: Docked image of beta secretase (PDB ID 1FKN) with human amyloid precursor protein E2 domain (PDB ID 3NYL).

6.5 SCREENING OF SELECTED COMPOUNDS BASED ON BINDING ENERGY:

Further we have screened compound that have energy greater than target enzyme-substrate complex and also they bind to the active site of beta secretase, to identify potential inhibitors of beta secretase that prevent formation of amyloid beta peptide, main cause of Alzheimer's disease. Selected compound that have energy greater than target enzyme-substrate complex have higher affinity to bind with the target beta secretase more than normal amyloid beta precursor protein and it also bind to the active site of beta secretase.

Table 5: illustrate binding mode and binding energy of compound having energy greater than target- substrate complex.

Sr. no.	Name of compound	Binding energy	Binding site
1.	PANTETHINE	-10.96	Bind near the active site
2.	SPERMINE	-10.92	Bind to both Asp 32 & Asp 228
3.	SPERMIDINE TRIHYDROCHLORIDE	-10.78	Bind to both Asp 32 & Asp 228
4.	CEPHALOSPORIN C SODIUM	-10.66	Bind near the active site
5.	CARNOSINE	-10.36	Bind to both Asp 32 & Asp 228
6.	LUNARINE	-10.26	Bind near the active site
7.	CYCLOSPORINE	-10.03	Bind near the active site
8.	CADAVERINE TARTRATE	-9.77	Bind to both Asp 32 & Asp 228
9.	PUTRESCINEDIHYDROCHLORIDE	-9.55	Bind to both Asp 32 & Asp 228
10.	ACARBOSE	-9.52	Bind near the active site
11.	MELEZITOSE	-9.42	Bind near the active site
12.	ORNITHINE	-9.38	Bind near the active site
13.	CYSTINE	-9.37	Bind to both Asp 32 & Asp 228
14.	AMYGDALIN	-9.23	Bind near the active site

15.	MUPIROCIN	-9.21	Bind near the active site
16.	3- HYDROXYTYRAMINE	-9.13	Bind to both Asp 32 & Asp 228
17.	SEROTONIN HYDROCHLORIDE	-9.04	Bind to both Asp 32 & Asp 228
18.	1R,2S PHENYLPROPYLAMINE	-9.00	Bind to both Asp 32 & Asp 228
19.	THIAMINE	-9.00	Bind to Asp 32

6.6 ANALYSIS OF THE DOCKING RESULT FOR IDENTIFICATION OF POTENTIAL INHIBITOR MOLECULE:

To identify potential inhibitors from the docked compound we have used ligplot. When we have opened the PDB file of docked molecule into ligplot it gives the mode of interaction like hydrogen binding, hydrophobic interaction, VANDERWALL forces etc and residues involved in the binding between target beta secretase and ligand molecule. starting that we can identify that the residues that bind to the active site or near the active site to target bate secretase protein can prevent the interaction between target protein and amyloid beta precursor protein by which the production of amyloid beta peptide stop. Here we have selected top five compounds that have binding energy more than target enzyme- substrate complex and they bind at the active site to the both residues of Asp 32 and Asp 228 of beta secretase. So these compounds may be effective inhibitors of beta secretase to prevent formation of amyloid beta peptide, responsible for the main cause of Alzheimer's disease.

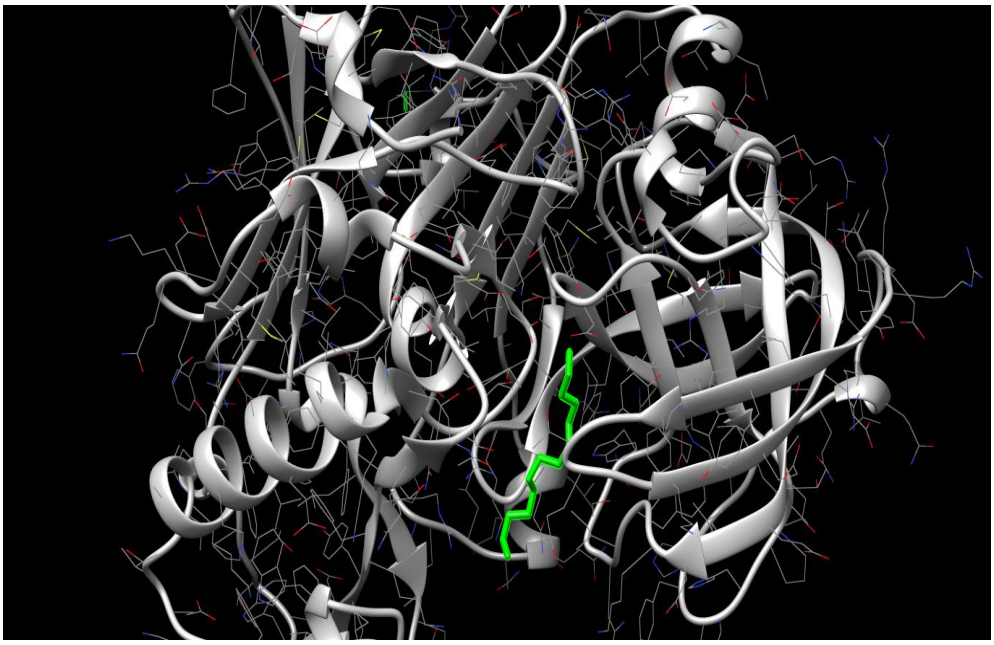


Figure 10: Shows docked image of SPERMINE with beta secretase (pdb.id. 1fkn).

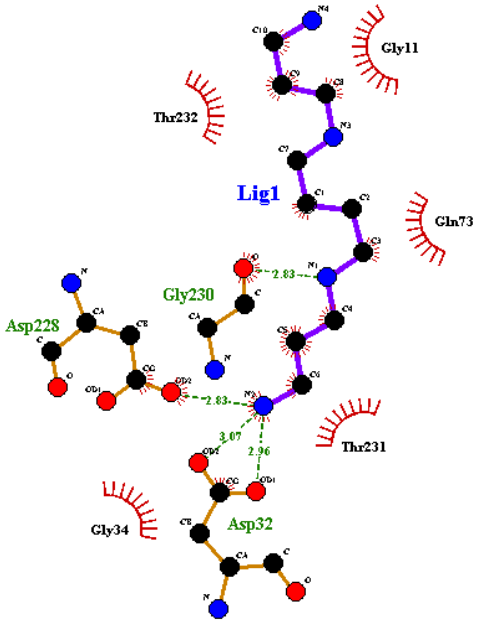


Figure 11: Interaction of SPERMINE molecule with beta secretase (pdb.id. 1fkn) had shown by ligplot.

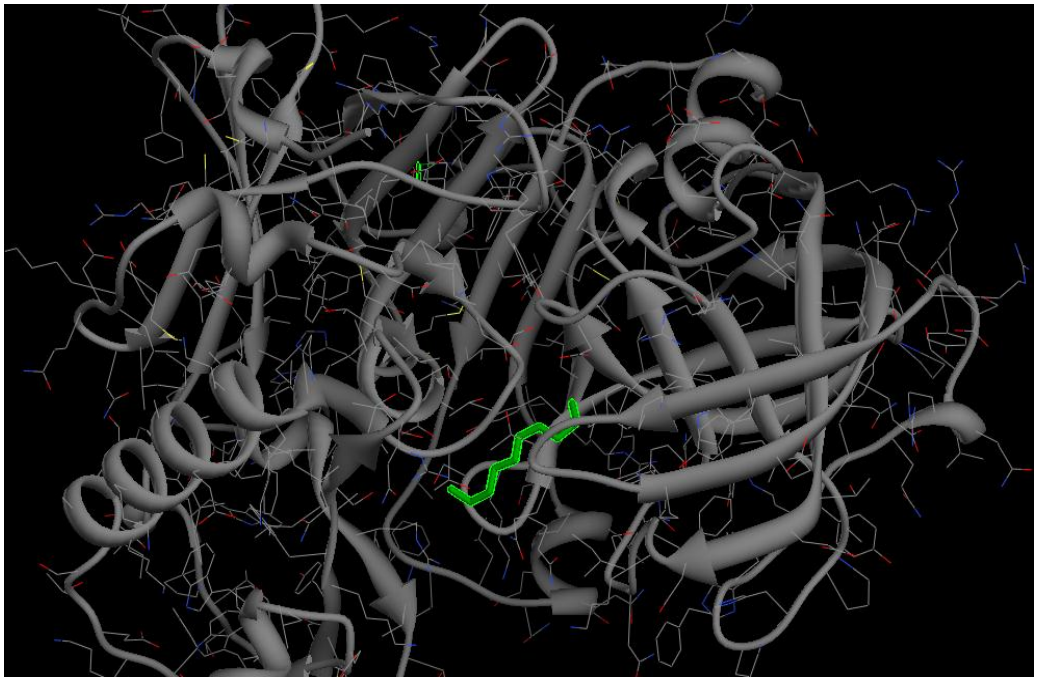
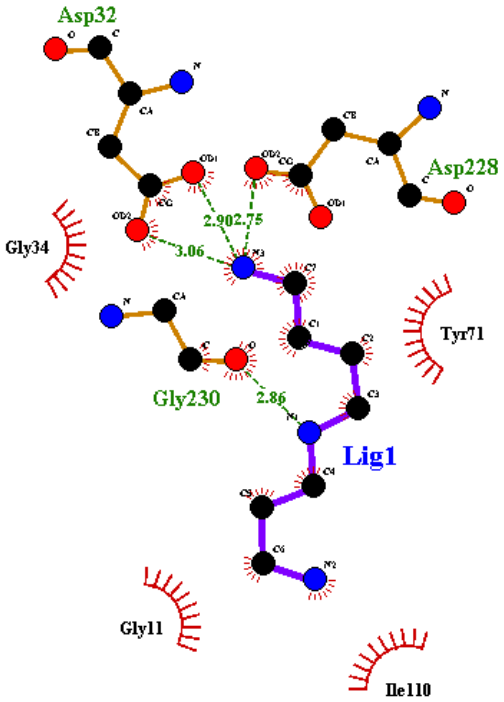


Figure 12: Shows docked image of SPERMIDINE TRIHYDROCHLORIDE with beta secretase (pdb.id. 1fkn).



SPERMIDINE TRIHYDROCHLORIDE

Figure 13: Interaction of SPERMIDINE TRIHYDROCHLORIDE molecule with beta secretase (pdb.id. 1fkn) has shown by ligplot.

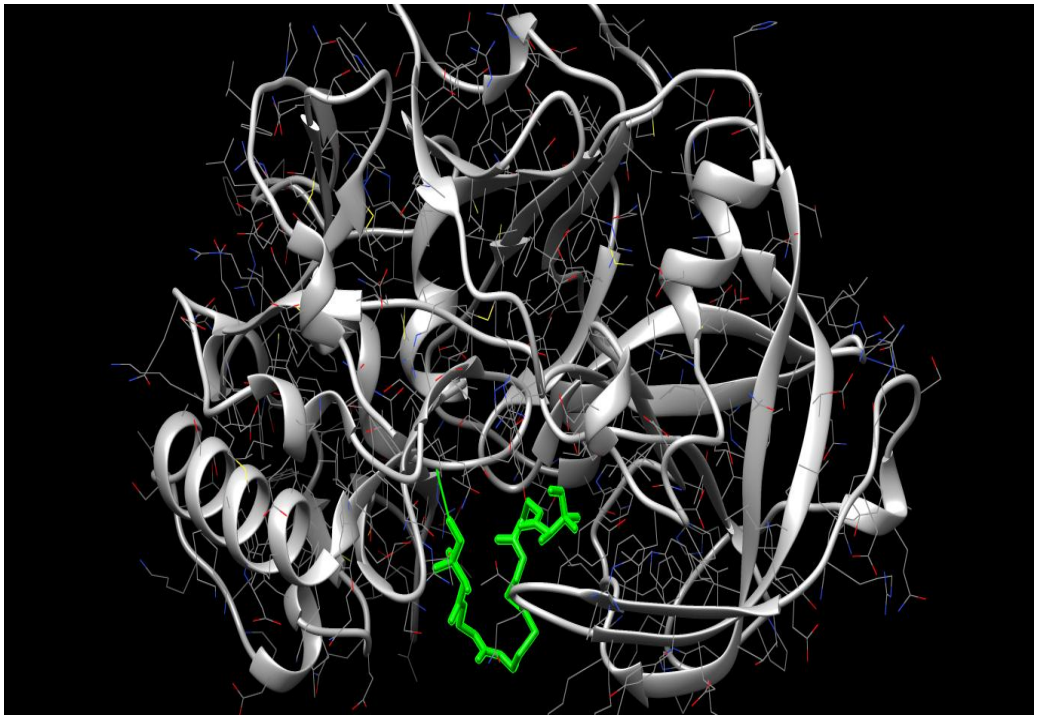
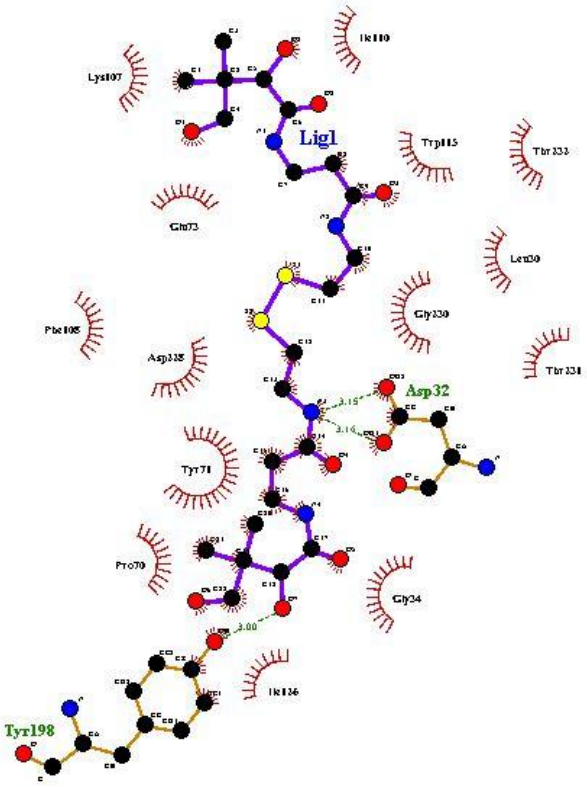


Figure 14: Shows docked image of PANTETHINE with beta secretase (pdb.id. 1fkn).



PANTETHINE

Figure 15: Interaction of PANTETHINE molecule with beta secretase (pdb.id. 1fkn) has shown by ligplot.

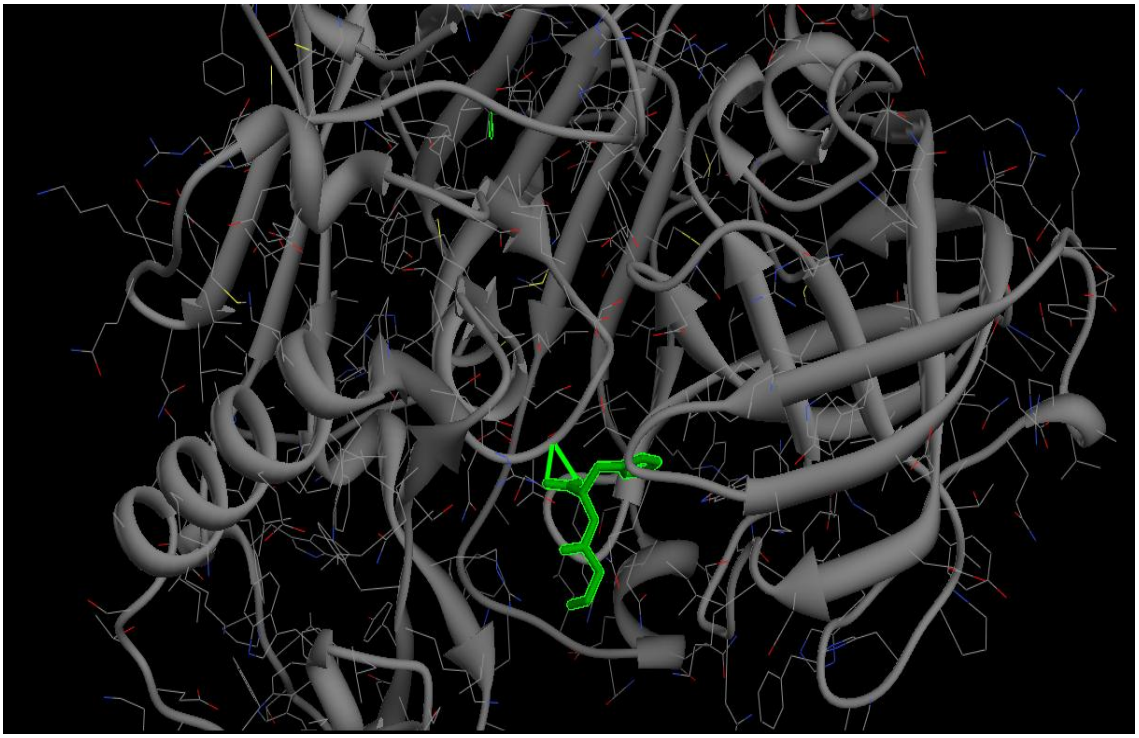


Figure 16*:* Shows docked image of CARNOSINE with beta secretase (pdb.id. 1fkn).

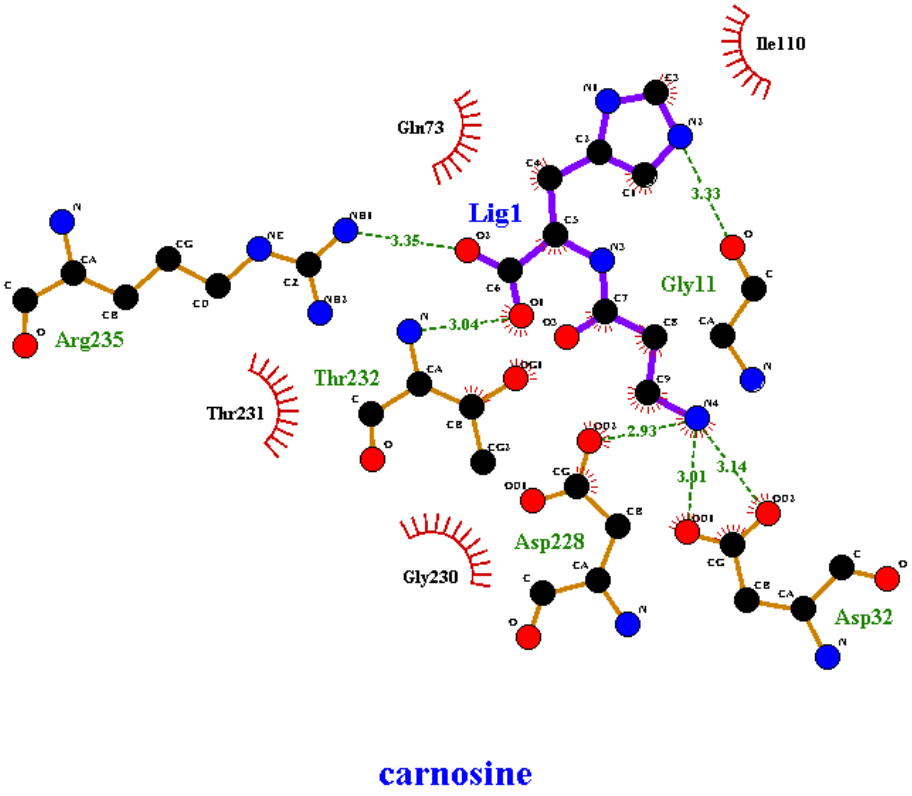


Figure 17*:* Interaction of CARNOSINE molecule with beta secretase (pdb.id. 1fkn) has shown by ligplot.

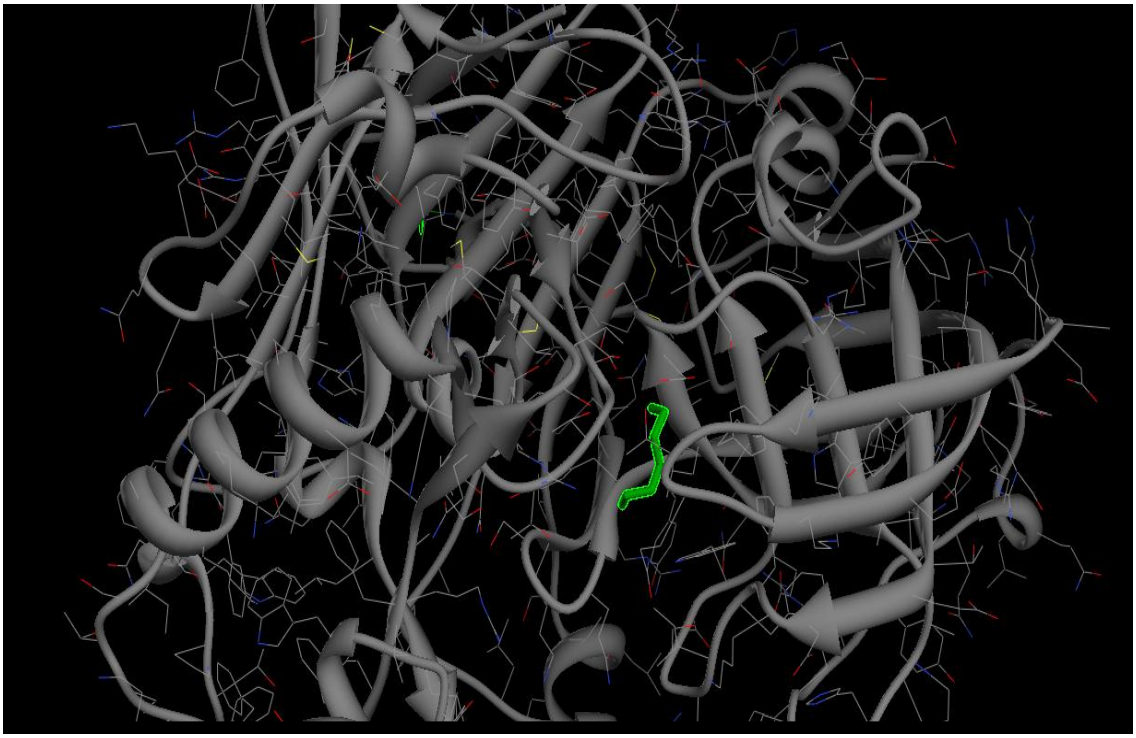


Figure 18: Shows docked image of CADAVERINE TARTRATE with beta secretase (pdb.id. 1fkn).

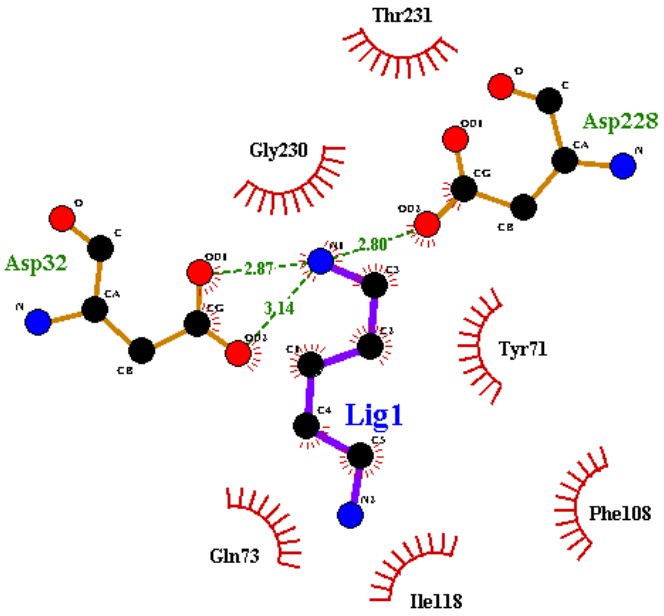


Figure 19: Interaction of CADAVERINE TARTRATE molecule with beta secretase (pdb.id. 1fkn) has shown by ligplot.

CHAPTER 7

DISCUSSION

7 DISCUSSIONS:

Alzheimer's disease is a most common amyloid-associated disorder, unalterable, progressive brain disorders that leads to alter in nerve cells destroying the assessment, remembrance, identification, interpretation and skills of persons. The symptom of Alzheimer's, disease generally appears in the persons having age more than 65 years. Alzheimer's disease uncomplaining have Insoluble, extracellular amyloid plaques, consist of fibrillar aggregates of the amyloid-beta (Aβ) peptide, a result of the proteolytic cleavage of β-amyloid precursor protein (APP). The Alzheimer's disease pathological hallmarks consist of existence of extracellular senile plaques and intracellular neurofibrillary tangles (NFT).Because neurofibrillary tangles are intracellular fibrillar aggregates of the microtubule-associated protein tau that demonstrate oxidative modifications and hyperphosphorylation. The drugs those are accessible for the treatments of Alzheimer's disease have limitations like they have low usefulness, have high charge and have brutal side effects. The two enzymes, γ-secretase and β-secretase (BACE-1 or Memapsin-2), are accountable for the sequential cleavage of APP for the creation of Aβ peptide. The cleavage of APP by BACE-1 leads to construction of two peptide fragments Ab40 and Ab42. Ab42 is considered to be accountable for the neurotoxicity and amyloid plaque creation in Alzheimer's disease (AD). Therefore BACE-1 will be a significant drug article for the production of inhibitors that lower Aβ. We aspire to recognize prospective natural product inhibitors of beta secretase which can be additionally developed as drug to care for Alzheimer's disease. In this study we performed in silico virtual screening approach of a natural product database consists of 800 diverse chemical molecules. Beta secretase (PDB ID: 1FKN) was used in screening procedure, and docking studies to identify potential lead

compounds. The molecules were selected based on their binding energy exposed that they have higher affinity to bind with target enzyme beta secretase. Active site of beta secretase is made up of two aspartate residues located as position 32 and 228 of the amino acid sequence of protein. Active site/ binding site of beta secretase were predicted by METAPOCKET 2.0 servers and DOGSITE scorer server. Binding site of beta secretase was also predicted by DOGSITE scorer, to verify protein druggability. DoGSite predicted that beta secretase is made up of 13 diverse pockets (binding site) and 10 dissimilar sub pockets, and give a Simple Score for every pocket between zero and one to predict druggability. According to this prediction the molecule that has score close to one having the property to be worked as potential inhibitors of beta secretase. So ligand molecules that have score close to one and those bind to the active site or near the active site can be serving as potential inhibitors of beta secretase. Bate secretase (PDB ID 1FKN) were docked with amyloid beta precursor protein E2 binding domain (PDB ID 3NYL) by using hex 6.1 tool, and binding energy calculated by hex was – 8.84. Further we have selected compound that have binding energy greater than binding energy of target- substrate complex and that bind to the active site or near the active site may work as a potential inhibitors of beta secretase.potential inhibitors from the docked compound was identified by using ligplot. From that we can identify that the residues that bind to the active site or near the active site to target bate secretase protein can prevent the interaction between target protein and amyloid beta precursor protein by which the production of amyloid beta peptide stop. At last we have selected top five compounds that have binding energy more than target enzyme- substrate complex and they bind at the active site to the both residues of Asp 32 and Asp 228 of beta secretase. Docking analysis has revealed involvement of hydrogen and hydrophobic interactions between the potential inhibitor molecules and the target

enzyme. Finally based on the binding energy and binding sites we have identified SPERMINE, SPERMIDINE TRIHYDROCHLORIDE, PANTETHINE, CARNOSINE, and CADAVERINE TARTRATE to be the top potential inhibitors against beta secretase. So these compounds may be effective inhibitors of beta secretase to prevent formation of amyloid beta peptide, responsible for the main cause of Alzheimer's disease. So the potential inhibitors may be SPERMINE, SPERMIDINE TRIHYDROCHLORIDE, PANTETHINE, CARNOSINE, and CADAVERINE TARTRATE molecules selected from natural product database.

CHAPTER 8

CONCLUSION

8 CONCLUSIONS

Alzheimer's disease is a most common amyloid-associated disorder, unalterable, progressive brain disorders that leads to alter in nerve cells destroying the thinking, remembrance, identification, interpretation and skills of persons. The neuropathological hallmarks of AD consist of occurrence of senile plaques (NP) and neurofibrillary tangles (NFT). Amyloid Beta precursor protein cleaving enzyme (BACE-1) belongs to the Aspartyl protease family, and it is accountable for the processing of the amyloid Precursor protein (APP). The cleavage of APP by BACE-1 leads to creation of two peptide fragments Ab40 and Ab42. Ab42 is deliberation to be accountable for the neurotoxicity and amyloid plaque formation in Alzheimer's disease (AD). In this study we have done virtual screening of compound retrieved from natural product database and docking study with beta secretase revealed that the natural compound having highest binding energy and bind to or near the active site can block the enzyme to bind with the substrate and thus work as a potential inhibitors of beta secretase for the treatment of Alzheimer's disease. Docking analysis has revealed involvement of hydrogen and hydrophobic interactions between the potential inhibitor molecules and the target enzyme. Finally based on the binding energy and binding sites we have identified SPERMINE, SPERMIDINE TRIHYDROCHLORIDE, PANTETHINE, CARNOSINE, and CADAVERINE TARTRATE to be the top potential inhibitors against beta secretase.

CHAPTER 9

FUTURE WORK

9 FUTURE WORKS

In this all above written conclusion are recognized by the assist of hopeful, consistent equipment and software of computational biology. In this current period of Insilco, each and every work is initially checked by Virtual screening or Insilico designing, after that we do invitro and invivo investigation. The future work of this study will be to do molecular dynamic simulation of the beta secretase inhibitors compound to study its behavior in real system. In vitro and in vivo validation will be required so that the selected inhibitors can be used as a potential drug candidate for inhibition of amyloid beta peptide formation which is the main cause of Alzheimer's disease.

CHAPTER 10

REFERENCES

10 REFERENCES

1. Schellenberg, G.D., et al., *The genetics of Alzheimer's disease.* Biomedicine & Pharmacotherapy, 1989. **43**(7): p. 463-468.
2. Armstrong, R.A., et al., *Relationships between β-amyloid (Aβ) deposits and blood vessels in patients with sporadic and familial Alzheimer's disease.* Neuroscience Letters, 1996. **207**(3): p. 171-174.
3. Coughlan, C.M. and K.C. Breen, *Factors influencing the processing and function of the amyloid β precursor protein—a potential therapeutic target in Alzheimer's disease?* Pharmacology & Therapeutics, 2000. **86**(2): p. 111-144.
4. Citron, M., *Emerging Alzheimer's disease therapies: inhibition of β-secretase.* Neurobiology of Aging, 2002. **23**(6): p. 1017-1022.
5. Graham, J.G. and N.R. Farnsworth, *3.04 - The NAPRALERT Database as an Aid for Discovery of Novel Bioactive Compounds*, in *Comprehensive Natural Products II*, M. Editors-in-Chief: Lew and L. Hung-Wen, Editors. 2010, Elsevier: Oxford. p. 81-94.
6. Willem, M., S. Lammich, and C. Haass, *Function, regulation and therapeutic properties of β-secretase (BACE1).* Seminars in Cell & Developmental Biology, 2009. **20**(2): p. 175-182.
7. Friedman, R., K. Boye, and K. Flatmark, *Molecular modelling and simulations in cancer research.* Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 2013. **1836**(1): p. 1-14.
8. Dickson, T.C. and J.C. Vickers, *The morphological phenotype of β-amyloid plaques and associated neuritic changes in Alzheimer's disease.* Neuroscience, 2001. **105**(1): p. 99-107.

9. Vogels, O.J.M., et al., *Cell loss and shrinkage in the nucleus basalis Meynert complex in Alzheimer's disease.* Neurobiology of Aging, 1990. **11**(1): p. 3-13.

10. Fraser, P.E., L. Lévesque, and D.R. McLachlan, *Biochemistry of Alzheimer's disease amyloid plaques.* Clinical Biochemistry, 1993. **26**(5): p. 339-349.

11. Yates, E.A., et al., *Specific Domains of Aβ Facilitate Aggregation on and Association with Lipid Bilayers.* Journal of Molecular Biology, (0).

12. Mattson, M.P., *Perturbed calcium homeostasis in neurodegenerative disorders*, in *Advances in Cell Aging and Gerontology*, P.M. Mark, Editor 2001, Elsevier. p. 29-54.

13. Arbus, C., et al., *Symptômes dépressifs dans la maladie d' Alzheimer :résultats préliminaires de l'étude REAL.FR.* La Revue de Médecine Interne, 2003. **24, Supplement 3**(0): p. 325s-332s.

14. White, A.R., et al., *Diverse fibrillar peptides directly bind the Alzheimer's amyloid precursor protein and amyloid precursor-like protein 2 resulting in cellular accumulation.* Brain Research, 2003. **966**(2): p. 231-244.

15. Lim, K.H., et al., *Characterizations of distinct amyloidogenic conformations of the Aβ (1–40) and (1–42) peptides.* Biochemical and Biophysical Research Communications, 2007. **353**(2): p. 443-449.

16. Iwata, N., M. Higuchi, and T.C. Saido, *Metabolism of amyloid-β peptide and Alzheimer's disease.* Pharmacology & Therapeutics, 2005. **108**(2): p. 129-148.

17. Chong, Y.H. and Y.-H. Suh, *Amyloidogenic processing of Alzheimer's amyloid precursor protein in vitro and its modulation by metal ions and tacrine.* Life Sciences, 1996. **59**(7): p. 545-557.

18. Pimplikar, S.W., *Reassessing the amyloid cascade hypothesis of Alzheimer's disease.* The International Journal of Biochemistry & Cell Biology, 2009. **41**(6): p. 1261-1268.

19. Mann, D.M.A., et al., *Saccharides of senile plaques and neurofibrillary tangles in Alzheimer's disease.* Neuroscience Letters, 1988. **85**(2): p. 277-282.

20. Gong, E.J., et al., *Morin attenuates tau hyperphosphorylation by inhibiting GSK3β.* Neurobiology of Disease, 2011. **44**(2): p. 223-230.

21. Guerreiro, R.J., et al., *Genetic screening of Alzheimer's disease genes in Iberian and African samples yields novel mutations in presenilins and APP.* Neurobiology of Aging, 2010. **31**(5): p. 725-731.

22. Masliah, E., R. Terry, and G.r. Buzsa´ki, *Thalamic nuclei in Alzheimer disease: evidence against the cholinergic hypothesis of plaque formation.* Brain Research, 1989. **493**(2): p. 240-246.

23. Chin, P.C., N. Majdzadeh, and S.R. D'Mello, *Inhibition of GSK3β is a common event in neuroprotection by different survival factors.* Molecular Brain Research, 2005. **137**(1–2): p. 193-201.

24. Samudralwar, D.L., et al., *Elemental imbalances in the olfactory pathway in Alzheimer's disease.* Journal of the Neurological Sciences, 1995. **130**(2): p. 139-145.

25. Ghosh, A.K., et al., *Potent memapsin 2 (β-secretase) inhibitors: Design, synthesis, protein-ligand X-ray structure, and in vivo evaluation.* Bioorganic & Medicinal Chemistry Letters, 2008. **18**(3): p. 1031-1036.

26. Findeis, M.A., *The role of amyloid β peptide 42 in Alzheimer's disease.* Pharmacology & Therapeutics, 2007. **116**(2): p. 266-286.

27. Citron, M., *β-Secretase inhibition for the treatment of Alzheimer's disease – promise and challenge.* Trends in Pharmacological Sciences, 2004. **25**(2): p. 92-97.

28. Ghosh, A.K., et al., *Structure-based design, synthesis, and biological evaluation of dihydroquinazoline-derived potent β-secretase inhibitors.* Bioorganic & Medicinal Chemistry Letters, 2012. **22**(17): p. 5460-5465.

29. Weiner, M.W., et al., *The Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception.* Alzheimer's & Dementia, 2012. **8**(1, Supplement): p. S1-S68.

30. Calabria, M., et al., *Efficacy of acetyl-cholinesterase-inhibitor (ACHEI) treatment in Alzheimer's disease: A 21-month follow-up "real world" study.* Archives of Gerontology and Geriatrics, 2009. **49**(1): p. e6-e11.

31. Chen, M.J., et al., *Gene profiling identifies commonalities in neuronal pathways in excitotoxicity: Evidence favouring cell cycle re-activation in concert with oxidative stress.* Neurochemistry International, 2013. **62**(5): p. 719-730.

32. Reynolds, G.P. and S.L. Kirk, *Metabolic side effects of antipsychotic drug treatment – pharmacological mechanisms.* Pharmacology & Therapeutics, 2010. **125**(1): p. 169-179.